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On

ELECTROMAGNETIC ACTIVATION OF GENE EXPRESSION AND CELL  
GROWTH

by

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and

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**ELECTROMAGNETIC ACTIVATION OF GENE EXPRESSION AND CELL  
GROWTH**

This application is based on, and claims the benefit of, U.S. Provisional Application No. 60/\_\_\_\_\_  
5 (yet to be assigned), filed January 22, 2003, which was converted from U.S. Serial No. 10/350,313, and entitled ELECTROMAGNETIC ACTIVATION OF GENE EXPRESSION AND CELL GROWTH, and which is incorporated herein by reference.

**BACKGROUND OF THE INVENTION**

10 This invention relates generally to methods for modulating the activity of gene products in a cell and, more specifically, to methods for modulating the activity of gene products that regulate tissue repair and cell proliferation by delivering electromagnetic energy to  
15 cells.

The normal development of all multicellular organisms relies on the orchestrated regulation of when and where each cell proliferates. For example, the  
20 formation of the intricate anatomical features of internal organs or the proper migration of nerves throughout the body require that each participating cell sense its environment and respond appropriately to developmental cues. The requirement for regulated  
25 proliferation is equally important for the proper functioning of the mature multicellular organism. The average adult human eradicates 50-70 billion cells in the body each day, and a commensurate number of replacement cells must be produced daily. The number and type of

cells that are induced to proliferate as replacements depends upon the circumstances under which the original cells were eradicated and the tissues affected.

5                   Harnessing the body's ability to regulate spatial and temporal aspects of cell proliferation is one approach to treating diseases and conditions characterized by traumatic or pathogenic tissue destruction. Growth factors have been considered  
10 candidate therapeutics for treating a number of such conditions because they are synthesized by and stimulate cells required for tissue repair, and are deficient in a number of chronic conditions. With the understanding that defects in growth factor signaling contribute to the  
15 development and/or persistence of a number of chronic conditions, it is logical to conclude that reinstitution or normalization of that signaling would promote healing. Although there is some evidence that pharmacological application of growth factors enhances healing in some  
20 conditions such as wound repair, it is often difficult to achieve targeted delivery of growth factors in such a way that healthy tissues are not inadvertently stimulated.

                  In particular, clinical studies of growth  
25 factor use in wound repair have been disappointing. The lack of therapeutic efficacy may be in part due to the complexity of the programmed sequence of cellular and molecular events involved in wound healing, including macrophage activation during inflammation, cell  
30 migration, angiogenesis, provisional matrix synthesis, synthesis of collagen by fibroblasts, and re-

epithelialization. Similarly complex sequences of cellular events are invoked during the repair of damage to tissues in response to other diseases and conditions. Current pharmaceutical approaches do not fully mimic the 5 necessary spatial and temporal patterns of cellular regulation and activity needed to promote cell proliferation for healing in most biological contexts.

The ability to control cell proliferation is 10 also important for growth of cells in culture for applications such as bioindustrial processing. Cultures of genetically engineered animal cells are currently used to produce post-translationally modified and physiologically active proteins for use as pharmaceutical 15 agents. Cell culture for pharmaceutical protein production in many cases is an expensive, slow process due to the complex media required and the slow rate of cell proliferation. Animal cells usually require mitogenic stimulation to proliferate. This mitogenic 20 stimulation is often provided by growth factors, which are supplied to the medium either as purified proteins or by the addition of animal blood sera.

The use of animal blood sera as a mitogen 25 causes a number of problems but nevertheless is used currently in biotechnological manufacturing processes employing animal cells. There is a risk that fetal blood sera will contain unwanted biological agents such as viruses, mycoplasma and prions, which if not properly 30 removed or avoided can contaminate the final pharmaceutical preparation and infect a patient. The screening of animal blood sera for viruses and mycoplasma

is feasible but expensive and complicated. Furthermore, inactivation of these contaminants by heating the serum often comes at the cost of inactivating valuable growth factors.

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The use of purified growth factor proteins as mitogens in cell culture, although providing advantages over the use of animal blood sera, is out of reach for many systems. The number and type of growth factors that 10 stimulate a particular animal cell to grow are not known in many cases. Even in cases where a useful growth factor has been identified, purified preparations are often required in large quantities. In this regard, 10,000 liter reactors are not unusual for the culture of 15 mammalian cells producing therapeutic proteins. The time and resources required to produce sufficient amounts of growth factors to sustain reactor cultures at these levels can be prohibitive.

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Thus, there exists a need for methods of stimulating cell proliferation and associated cellular processes *in vivo* and *in vitro*. The present invention satisfies this need and provides related advantages as well.

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#### SUMMARY OF THE INVENTION

The invention is directed to a method for accelerating the cell cycle by delivering to a cell an effective amount of electromagnetic energy. The 30 invention also provides a method for activating a cell cycle regulator by delivering to a cell an effective

amount of electromagnetic energy. Also provided by the invention is a method for activating a signal transduction protein; a method for activating a transcription factor; a method for activating a DNA synthesis protein; and a method for activating a Receptor. A method for inhibiting an angiotensin receptor as well as a method for reducing inflammation also are provided by the present invention. The invention also is directed to a method for replacing damaged neuronal tissue as well as a method for stimulating growth of administered cells.

**BRIEF DESCRIPTION OF THE DRAWINGS**

15           Figure 1 shows the growth stimulation of untreated human dermal fibroblasts (HDF) by media transferred from HDF cells exposed to electromagnetic energy. Figure 1A shows the growth response of the HDF cells from which the medium was transferred from at the 20 hours shown. Figure 1B shows the induction of proliferation of untreated cells to which the media from the cells in Figure 1A was transferred.

25           Figure 2 shows a Western blot that demonstrates significant activation of ERK-1 (p44) and ERK-2 (p42) after the initiation of treatment with electromagnetic energy.

30           Figure 3 shows incorporation of BrdU as an indicator of entry into S phase of HDF cells stimulated with electromagnetic energy.

Figure 4 shows two autographs that show gene expression in human diploid fibroblasts. Each array contains 1,176 known cDNA sequences involved in tissue repair, cell cycle and cell growth. The Black arrows are 5 examples of genes that are not increased in expression following treatment with electromagnetic energy. The Grey arrows are examples of genes significantly up-regulated in treated cells. The brackets at the bottom of the arrays indicate control cDNA sequences used to 10 normalize samples.

Figure 5 shows autographs of the analysis of cDNA sequences implicated in inflammation processes. Each array contains 234 cDNAs in duplicate. The Black arrows 15 are examples of genes that are not increased in expression following electromagnetic treatment. The Grey arrows are examples of genes significantly up-regulated in Provant treated cells. The brackets at the bottom of the arrays indicate control cDNA sequences used to 20 normalize samples.

Figure 6 shows expression profiles of genes in HDF cells treated with electromagnetic energy. In Figure 6A the genes representing the entire set of fibroblast 25 microarray data are grouped into clusters representing similarity of expression patterns, regardless of function. The functional groupings in panels 6B-6D represent genes selected from groups of genes whose function is important to cell division and/or wound 30 healing. Both sets of data are arranged so that early expression genes are displayed first, followed by

intermediate expression and late expression. The scale in each panel (0 to 8) represents the ratio of the raw expression level for the experimental time point to the expression level in a non-treated control scenario. For 5 example, dark shading means an eight-fold induction over control. Figure 6B shows expression levels of genes divided into the following functional groups: Adhesion Molecules; Cyclins; DNA Synthesis Proteins; and Growth Factors and corresponding Receptors. Figure 6C shows 10 expression levels of genes divided into the following functional groups: Interleukins, Interferons and corresponding Receptors; MAP Kinases; other kinases; and Matrix Metalloproteinases and their Inhibitors. Figure 6D shows expression levels of genes divided into the 15 following functional groups: Protein Kinase Cs; Tumor Necrosis Factors and their Receptors; and Transcription Factors. Measurements for all genes were for expression between 5 minutes and eight hours post-treatment.

20 Figure 7 shows expression profiles of genes in human keratinocytes treated with electromagnetic energy. In Figure 7A the genes representing the entire set of keratinocyte microarray data are grouped into clusters representing similarity of expression patterns, 25 regardless of function. The functional groupings in panels 7B-7D represent genes selected from groups of genes whose function is important to cell division and/or wound healing. Both sets of data are arranged so that early expression genes are displayed first, followed by 30 intermediate expression and late expression. The scale in each panel (0 to 8) represents the ratio of the raw expression level for the experimental time point to the

expression level in a non-treated control scenario. For example, dark shading means an eight-fold induction over control. Figure 7B shows expression levels of genes divided into the following functional groups: Adhesion Molecules; Cyclins; DNA Synthesis Proteins; and Growth Factors and corresponding Receptors. Figure 7C shows expression levels of genes divided into the following functional groups: Interleukins, Interferons and corresponding Receptors; MAP Kinases; other kinases; and Matrix Metalloproteinases and their Inhibitors. Figure 7D shows expression levels of genes divided into the following functional groups: Protein Kinase Cs; Tumor Necrosis Factors and their Receptors; and Transcription Factors. Measurements for all genes were for expression between 5 minutes and eight hours post-treatment.

#### DETAILED DESCRIPTION OF THE INVENTION

The present invention is based on the discovery that stimulation of cells with electromagnetic energy modulates the activity of genes involved in tissue repair and cell growth and the cellular levels of gene products that are involved in molecular regulatory networks. As demonstrated herein, stimulation with electromagnetic energy modulates the levels of gene products such as extracellular matrix receptors, signal transduction proteins, cell cycle regulators, transcription factors and nucleic acid synthesis proteins. The changes to these regulatory networks lead to changes in cellular functions that include, but are not limited to, acceleration of the cell cycle, stimulation of wound

healing, stimulation of cell proliferation, stimulation of tissue growth, and modulation of inflammatory responses. Accordingly, the invention provides methods for delivering to a cell an effective amount of 5 electromagnetic energy to change such cellular functions. Furthermore, the invention provides diagnostic methods for monitoring the cell cycle, wound healing, tissue growth, or inflammation by determining a level of a gene product involved in a regulatory network.

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The invention is further based on the discovery that delivery of electromagnetic energy to a resting cell accelerates the cell cycle, not only by inducing entry 15 into the cell cycle, but also by reducing duration of the cell cycle. In particular, the gap,  $G_1$  phase, that intervenes between the formation of a daughter cell by mitosis,  $M$  phase, and DNA synthesis,  $S$  phase, is shortened by delivering electromagnetic energy in accordance with a method of the invention. Accordingly, 20 the invention provides a method for accelerating the cell cycle of a population of cells by delivering electromagnetic energy to the population of cells. In particular embodiments, a method for stimulating proliferation of a population of cells can be used *in* 25 *vitro*, for example, to produce replacement tissues, or *in vivo*, for example, to stimulate introduction of therapeutic cells or to stimulate replacement of damaged cells such as at the site of a wound.

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As used herein, the term "cell cycle" is intended to mean the process of cell replication occurring between the formation of a cell by division

from its mother cell and its division to form two daughter cells. The cell cycle can be divided into a number of periods typically identified as M phase, which is the period of mitosis and cell division; G<sub>1</sub>, which is 5 the gap period occurring after telophase of mitosis and prior to S phase; S phase, which is the period of DNA synthesis occurring after G<sub>1</sub> and before G<sub>2</sub>; and G<sub>2</sub>, which is the gap period after S phase and before prophase of mitosis.

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As used herein, the term "accelerate," when used in reference to the cell cycle, is intended to mean decreasing the period of time for the cell cycle in a replicating cell. A replicating cell is a cell that is 15 in M, G<sub>1</sub>, S or G<sub>2</sub> phase. In contrast, a non-replicating cell is a cell that is in the resting phase known as G<sub>0</sub> phase. A decrease in the period of time can include a decrease in the period of time spent in the G<sub>1</sub>, G<sub>2</sub> or S phase.

20

As used herein, the term "electromagnetic energy" is intended to mean a form of energy having both electric and magnetic components and properties of wavelength and frequency. Forms of energy included in 25 the term are, for example, X-ray radiation, which has a wavelength in the range of about 0.05 to 100 angstroms; ultraviolet radiation, which has a wavelength in the range of about 200 to 390 nm; visible radiation, which has a wavelength in the range of about 391 to 770 nm; 30 infrared radiation, which has a wavelength in the range of about 0.771 to 25 microns; microwave radiation, which

has a wavelength in the range of about 1 millimeter to 1 meter; and radiofrequency radiation, which has a wavelength greater than about 1 meter.

5           As used herein, the term "cell cycle regulator" is intended to mean a molecule that activates or inhibits progression through the cell cycle. A molecule included in the term can activate progression through the cell cycle by initiating the cell cycle or a phase of the cell 10 cycle or by increasing the rate of the cell cycle or a phase of the cell cycle. A molecule included in the term can inhibit progression through the cell cycle by stopping the cell cycle or a phase of the cell cycle or by decreasing the rate of the cell cycle or a phase of 15 the cell cycle. Examples of molecules included in the term are cyclins such as Cyclin H; cyclin dependent kinases such as CDKN2D, CDK7, CDK5 and CDK6; CLK1; CKS2; LHX1; Cyclin 6 Kinase; Cell Cycle Regulated Kinase; CDK inhibitors and CDC20.

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As used herein, the term "signal transduction protein" is intended to mean a protein that converts input energy of one form to output energy of another form in a regulatory network of a cell. The term can include, 25 for example, a kinase, phosphatase, or G-protein. Other examples of proteins included in the term are MAP3K11, MAPK7/ERK5, MAPK5/MEK5, MEK1, MEK2, MEK3, MAP kinase p38, BDIIF Tyr Kinase, Serine Kinase, p68 Kinase, PAK2 and SPS1/ste20

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As used herein, the term "transcription factor" is intended to mean a protein that initiates or regulates

synthesis of RNA when in the presence of a DNA template and RNA polymerase. Examples of proteins included in the term include TFIIB 90-Kd, C-jun, Est1, and Early Response Protein.

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As used herein, the term "DNA synthesis protein" is intended to mean a protein that catalyzes or facilitates formation of a bond between nucleotides of a deoxyribonucleic acid polymer. Examples of proteins included in the term are helicases such as DNA Helicase A, ligases such as DNA Ligase 1, DNA Polymerases such as DNA Polymerase Delta, topoisomerases such as Topoisomerase I, and DNA Repair Enzymes.

15 As used herein, the term "receptor" is intended to mean a protein that binds to a molecule and transduces a signal that alters cell function. A protein included in the term can be a soluble protein or membrane protein. Examples of proteins included in the term are the  
20 Angiotensin Receptor, Tyrosine Kinase Receptor, Thrombin Receptor, Adenosine A1 Receptor, Na/H Exch, Ephrin A Receptor, Insulin Receptor, Cell-Cell Adhesion Protein, Matrix Adhesion Protein, ICAM1, H<sub>2</sub>O Channel, Integrin $\beta$ 8, K<sup>+</sup> Channel, Glucose Transporter, TGF $\beta$  Receptor, PDGF  
25 Receptor, Cl<sup>-</sup> Channel, TNF Receptor, IGFBP1, Ras Homolog, RAS Associated Protein, RAS GTPase, RAB6, RAB5A, Ca<sup>+2</sup> Adenylylcyclase, Adenylylcyclase, Protein Kinase C and S100 Ca<sup>+2</sup> Binding Protein.

30 As used herein, the term "activating," when used in reference to a gene product, is intended to mean

increasing the activity of the gene product. The activity can be increased, for example, by increasing the expression of the gene product, decreasing degradation of the gene product, increasing the catalytic rate of the 5 gene product or increasing affinity of the gene product for its substrate.

As used herein, the term "tissue" is intended to mean a group of cells united to perform a particular 10 function. A group of cells included in the term can further form an ordered structure such as a tube or sheet. Alternatively a group of cells can be unstructured, for example, occurring in mass or clump. Examples of tissues include epithelial, connective, 15 skeletal, muscular, glandular, and nervous tissues.

As used herein, the term "stimulating growth" is intended to mean initiating or increasing the rate at which cells proliferate. The term can include, for 20 example, accelerating the cell cycle, initiating entry into the cell cycle, or leaving  $G_0$  or the resting state.

As used herein, the term "wound" is intended to mean a stress to a tissue due to injury. A stress to a 25 tissue can involve a breach and included in the term can be a chronic wound, pressure ulcer, diabetic ulcer, venous stasis ulcer, burn or trauma. The term can include a breach that is at a particular stage of healing including, for example, an inflammatory phase in which 30 leukocytes migrate to the wound site and monocytes are converted to macrophages; proliferative phase in which granulation occurs due to proliferation of fibroblasts,

production of a collagen matrix and vascularization; epithelialization phase in which epithelial cells grow along fibrin and myofibroblasts synthesize collagen; or differentiation phase in which collagen is degraded and 5 resynthesized as the tissue is remodeled.

As used herein, the term "matrix" is intended to mean a substrate capable of supporting a population of proliferating cells. The term can include, for example, 10 a synthetic substrate or polymer such as nylon (polyamides), dacron (polyesters), polystyrene, polypropylene, polyacrylates, polyvinyl compounds (e.g., polyvinylchloride), polycarbonate (PVC), polytetrafluoroethylene (PTFE; teflon), thermanox (TPX), 15 nitrocellulose or polyglycolic acid (PGA). Also included in the term is a biological matrix such as cotton, cat gut sutures, cellulose, gelatin, dextran or an in vivo site such as a tissue or wound.

As used herein, the term "level," when used in reference to a molecule, is intended to mean an amount, concentration, or activity of the molecule. An amount or concentration included in the term can be an absolute value such as a molar concentration or weight or a 25 relative value such as a percent or ratio compared to one or more other molecules in a sample. An activity can be an absolute value such as a turnover number, reaction rate, or binding constant or a relative value such as a percent or ratio compared to one or more other molecules.

The invention provides a method for accelerating the cell cycle. The method includes a step of delivering to a cell an effective amount of electromagnetic energy to accelerate the cell cycle of 5 the cell.

The methods of the invention provide for acceleration of the cell cycle such that cells that are actively replicating do so at a faster rate. The cell 10 cycle is accelerated in the methods at least in part by a reduction in the duration of the G<sub>1</sub> stage of the cell cycle. When the cell cycle is accelerated for a replicating cell, the rate at which the cell completes the cell cycle and replicates its DNA is increased. 15 Generally, a population of cells can include cells that are replicating in the cell cycle, resting in G<sub>0</sub>, or a combination of cells in both states. For a population that includes resting cells in the G<sub>0</sub> state, growth of the population can be stimulated by inducing the resting 20 cells to enter the cell cycle and become replicating cells. A mixture of cells containing both resting and cycling cells can be stimulated and growth increased due to both acceleration of the cell cycle for cells that are replicating as well as recruitment of resting cells into 25 the cell cycle. However, acceleration of the cell cycle provides a different means of increasing the rate at which a population of cells grows compared to recruitment of cells into the cell cycle. As set forth in further detail below, acceleration of and recruitment into the 30 cell cycle can be induced in a method of the invention by modulating the activity of molecular regulatory networks controlling the cell cycle.

Acceleration of the cell cycle will result in a decrease in the period of time for the cell cycle of a treated cell compared to an untreated cell. An effective amount of electromagnetic energy can be delivered in accordance with the methods described herein to accelerate the cell cycle to achieve a desired rate of cell proliferation. In some applications of the methods an effective amount of electromagnetic energy can be delivered to cause a 10%, 25%, 50% or 75% increase in the cell cycle. When a faster rate of cell proliferation is desired, an effective amount of electromagnetic energy can be delivered resulting in, for example, a 2 fold, 3 fold, 4 fold, 5 fold or higher increase in the cell cycle. An untreated cell used for comparing a cell that has been contacted with electromagnetic energy can be any cell that is not influenced by treatment with electromagnetic energy including, for example, the cell itself prior to delivery of electromagnetic energy or a control cell that is not treated with an effective amount of electromagnetic energy to accelerate the cell cycle. The magnitude of cell cycle acceleration can be influenced by altering parameters of electromagnetic energy delivered in a method of the invention as set forth in further detail below.

Electromagnetic energy is delivered to a cell using any apparatus capable of generating and applying known dosages of electromagnetic energy of defined specifications to the cell. Generally, an apparatus useful in the invention for delivering electromagnetic

energy to a cell will include an electromagnetic energy generator, a treatment applicator that delivers energy from the generator to a cell and a device for controlling the amount or characteristics of the electromagnetic energy delivered by the applicator. An exemplary electromagnetic energy treatment apparatus that can be used in a method of the invention is described in U.S. Pat. No. 6,344,069 B1, which describes an apparatus that includes a pulsed electromagnetic energy generator; a power controller, including a power level controller responsive to signals from multiple sensing and control circuits; and a treatment pad applicator.

The parameters under which electromagnetic energy is delivered to a cell can be adjusted to suit a particular application of the methods. Exemplary parameters that can be adjusted include, without limitation, wavelength, power level, duration of delivery, delivery of constant output or pulsed output and, if pulsed output is used, pulse rate and pulse width. A power level in the range of about 1 to 300 mw/cm<sup>2</sup> (60 to 1,065 V/m) is useful in a method of the invention. The pulse rate can be any in the range of about 100-3,600 ppm (pulses per minute), while pulse width is typically in the range of about 5-300 microseconds. The wavelength or frequency of the electromagnetic energy can be in a range selected from x-ray radiation, ultraviolet radiation, visible radiation, infrared radiation, microwave radiation, radiofrequency radiation, or combination thereof. Typically, the electromagnetic energy is delivered under parameters in

which the cell being treated does not sustain substantial DNA damage.

As an exemplary application, parameters that

5 are effective for acceleration of the cell cycle for treatment of wounds include delivery of RF frequency energy with an average power of about  $15 \text{ mw/cm}^2$ ,  $32 \text{ mw/cm}^2$ , or  $100 \text{ mw/cm}^2$  (about  $240 \text{ V/m}$ ,  $350 \text{ V/m}$  or  $600 \text{ V/m}$ ) pulse envelopes with a duration of about 32 microseconds

10 and a repetition rate of about 1,000 pulses per second. For example, in treating pressure ulcers, power of the RF energy can about  $30-40 \text{ mw/cm}^2$  ( $335-390 \text{ V/m}$ ) with a pulse envelope having a duration between about 16-20 microseconds and a repetition rate between about 1,200-

15 1,500 pulses per second. In another effective embodiment, RF energy is delivered with a repetition rate in the range of about 900-1,200 pulses per second and a duration of about 30-45 microseconds, giving an output of in the range of about  $30-65 \text{ mw/cm}^2$  ( $335-500 \text{ V/m}$ ) average power.

20 In yet another embodiment, RF energy is delivered with a repetition rate in the range of about 600-1,000 pulses per second and a pulse duration in the range of about 32-60 microseconds, giving an output in the range of about  $30-100 \text{ mw/cm}^2$  ( $335-600 \text{ V/m}$ ) average power. Other

25 parameters useful in the invention are demonstrated in the Examples provided below. The parameters exemplified above with respect to wound healing can be used in other applications of the methods such as reducing inflammation, stimulating cell proliferation,

30 accelerating the cell cycle, modulating the activity of a gene product or replacing a damaged tissue.

Another parameter that can be adjusted is the number of electromagnetic energy deliveries given to a cell during a specified time period. Electromagnetic 5 energy can be delivered in a single administration or in multiple. Multiple deliveries can be administered over a time period of minutes, hours, days or weeks. For example, an effective treatment profile for wound healing is described in U.S. Pat. No. 6,344,069 B1 and includes 10 delivery of electromagnetic energy twice a day, eight to twelve hours between treatments, for thirty minutes per treatment.

The parameters for delivery of electromagnetic 15 energy for a particular application of the methods can be determined based on a dose-response analysis. Those skilled in the art will know or be able to determine an appropriate response that indicates a favorable outcome for a particular application such as treatment of a 20 disease or condition and will be able to systematically vary the parameters while evaluating the response as it correlates with a desired outcome. Exemplary diseases and conditions that can be treated using a method of the invention and responses that are indicative of a 25 favorable outcome are set forth in further detail below. A further response that can be monitored in a dose-response analysis is expression of particular genes or activity of gene products, which is also set forth in further detail below.

30

The invention provides a method for delivering an effective amount of electromagnetic energy to modulate

the activity of a cellular component. The activity of a cellular component can be modulated by increasing or decreasing the level of the cellular component in the cell, for example, by a change in expression level or 5 stability. Activity of a cellular component can also be modulated by a covalent modification of the molecule including, for example, addition of a phosphate by a kinase, removal of a phosphate by a phosphatase or addition or removal of other chemical moieties such as 10 complex carbohydrates or hydrocarbons like prenyl, farnesyl, or geranylgeranyl groups. Further modulation of cellular component activity can include increase or decrease in activity due to a change in a level of a substrate or inhibitor of the component.

15

Delivery of electromagnetic energy in a method of the invention can modulate the activity of cellular components including, without limitation, cell cycle regulators, signal transduction proteins, transcription 20 factors, DNA synthesis proteins or receptors. Examples of particular cellular components that can be activated or inhibited by delivery of electromagnetic energy in a method of the invention are set forth above in the definitions and in the Examples below. A regulatory 25 network to which an electromagnetically affected component belongs will be influenced by the change in activity. Accordingly, a method of the invention can be used to modulate the activity of a network to which a particular component belongs. Those skilled in the art 30 will know or be able to determine what networks are affected by a particular component and will be able to

determine how the network is affected based on the change in activity of the component as it relates to its function in the network. One or more components that have modulated activity in response to delivery of 5 electromagnetic energy can be used to monitor the effectiveness of treatment as set forth in further detail below.

Delivery of electromagnetic energy to a cell in 10 a method of the invention can be used to activate mitogenic signaling pathways. As demonstrated in Example 1, electromagnetic energy stimulates release of soluble factors via transduction pathways that include ERK-1. The soluble factors themselves provide mitogenic 15 stimuli to cells further activating mitogenic signaling pathways in a feed forward manner in the treated cells and additionally, stimulate mitogenic signaling pathways in other cells as well. Thus, delivery of electromagnetic energy to a cell can be used to modulate 20 the activity of components in mitogenic signaling pathways including, for example, those set forth below.

Several different classes of kinases associated with mitogenic stimuli are known and are referred to as 25 mitogen activated protein (MAP) kinases. MAP kinases can be classified into three types including extracellular signal regulated kinases (ERKs), c-Jun NH<sub>2</sub>-terminal kinases (JNKs), and p38 kinases. The latter two are often grouped together as stress activated protein 30 kinases (SAPKs). The two most predominant forms of ERK kinases are ERK-1 and ERK-2, also referred to as p44 and p42 MAP Kinases, respectively. They are ubiquitously

expressed in the body, and, within cells, can be found in the cytoplasm, nucleus, and associated with the cytoskeleton. ERK-1 and ERK-2 are activated in fibroblasts by serum, growth factors, cytokines, and in 5 some cases stress, although these pathways are typically considered non-stress pathways.

The JNK and p38 pathways are more traditionally associated with stress activation. JNKs can be activated 10 by cytokines, agents that interfere with DNA and protein synthesis, or other stresses. They can also be activated by serum and growth factors, although less frequently. The p38 kinases are activated by cytokines, hormones, osmotic and heat shock, as well as other stresses.

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While the JNK and p38 pathways are typically activated only by G-protein coupled receptors, the ERK pathway can be activated by both G-protein coupled receptors and tyrosine kinase receptors. The pathways 20 activated by these two classes of receptors are distinct, but tend to overlap further down the cascade. As a result, activation of G-protein coupled receptors can result in activation of pathways associated with both classes of receptors.

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G-protein coupled receptors are a broad group of receptors. They are involved in a wide variety of biological functions, including endocrine and exocrine regulation, exocytosis, platelet function, embryogenesis, 30 angiogenesis, tissue regeneration, and control of cell growth.

Different G-protein coupled receptors can interact with the ERK's through several different pathways. In general, the cascade is activated when a ligand binds to the receptor, causing a conformational change in the  $\alpha$  subunit of the G-protein. As a result of the conformational change, the  $\alpha$  subunit exchanges a GDP for a GTP, thereby becoming active and liberating the  $\beta\gamma$  heterodimer. Both the  $\alpha$  and the  $\beta\gamma$  subunits are able to activate ERK.

The diversity of interactions of the G-proteins with ERK pathways is primarily achieved through different varieties of  $\alpha$  subunits. The  $G\alpha_q$  subunit interacts with the ERK pathway by controlling PLC- $\beta$ , which hydrolyzes phosphatidylinositol 4,5-biphosphate to form IP<sub>3</sub> and diacylglycerol (DAG), both of which are upstream effectors of ERK. In contrast, the  $G\alpha_s$  subunit activates ERK by working through adenylyl cyclases, which generate cAMP, another upstream effector of ERK activation.  $G\alpha_I$ , on the other hand, inhibits activation of adenylyl cyclases. The activity of these and other  $\alpha$  subunits involved in the ERK pathways can be modulated by delivery of electromagnetic energy to a cell in which they are expressed. The  $\beta\gamma$  heterodimer also plays a separate role in ERK activation, for example, in the JNK pathway, the  $\beta\gamma$  subunit, along with  $\alpha_{12}$  and  $\alpha_{13}$  are the G-protein subunits primarily responsible for activation.

Downstream of the G-proteins, there are several different factors that are independently activated, depending on the  $G\alpha$  subunit involved. One such set of factors, important in both the ERK and JNK pathways, are 5 families of proteins known as GTPases. Associated with the ERK pathway is the Ras family of GTPases while associated with the JNK pathway is the Rho family. Ras is activated in response to the interactions of several proteins including, for example, Sos, a Ras-guanine 10 nucleotide exchange factor; Grb2, an adapter protein; and Shc, which is activated by the  $\beta\gamma$  subunit. Activation of Ras results in formation of Ras-GTP and occurs when Sos associates with Grb2 and Shc, an interaction that occurs in association with tyrosine kinase receptors. Rho 15 family proteins, including Rac1 and Cdc42, are activated by  $G\beta\gamma$ ,  $G\alpha_{12}$ , and  $G\alpha_{13}$ . Rac1 and Cdc42 in turn activate kinases upstream of JNK, such as PAK and MLK3/DLK.

As set forth above, the  $G\alpha_q$  subunit influences 20 the activity of PLC- $\beta$ , which cleaves phosphatidylinositol 4,5-biphosphate to form  $IP_3$  and DAG.  $IP_3$  and DAG work in concert to release intracellular stores of calcium and activate PKC. PKC activates Raf-1, a MAP Kinase Kinase Kinase, which also interacts with Ras.

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The  $G\alpha_s$  subunit activates the ERK pathway through interaction with adenylyl cyclases, of which there are at least 10 forms capable of generating cAMP. These forms of adenylyl cyclases are activated by  $G\alpha_s$ , 30 but they are differentially regulated by calcium,

phosphorylation,  $\beta\gamma$  subunits and  $\alpha$  inhibitory subunits. The changing concentrations of cAMP affect PKA activity in a cell-type dependent fashion. In fibroblasts and vascular smooth muscle cells, elevation of cAMP levels 5 causes inhibition of ERK activation by interfering with PKA's ability to activate Raf-1. However, in ovarian, pituitary and neuronal cells, among others, elevation of cAMP levels promotes ERK activity by inactivating Raf-1 and stimulating PKA to activate Rap1 and B-Raf.

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As set forth above, after the activation of G-proteins, activation can branch off in many different directions including, for example, along the Ras pathway, the PKA pathway, or the PKC pathway. These separate 15 interactions are part of a greater network wherein the pathways influence each other, for example, through regulation of the activity of Rap1 and the Rafs. Rap1 is another GTPase, which, as set forth above, is activated by PKA. There are at least three forms of Raf including 20 Raf-1, A-Raf, and B-Raf. Both B-Raf and Raf-1 can be activated by PKA, while only Raf-1 is activated by PKC. On the other hand, activation of Raf-1 by PKA can also be inhibited by high concentrations of cAMP, depending on cell type, as set forth above.

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Interactions among Ras, Rap1 and the three forms of Raf influence multiple signal transduction pathways thereby acting as nodes connecting these pathways in a larger signal transduction network. 30 Activation of all three Rafs requires the presence of active Ras, although only B-Raf can be activated solely by Ras. Rap1 can either stimulate or inhibit ERK

activation. This is dependent on whether it is interacting with B-Raf, in which case it stimulates ERK activation, or Raf-1 and A-Raf, in which case it inhibits ERK activation.

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The Rafs have MAP Kinase Kinase Kinase activity, and are referred to as MAPK/ERK Kinase Kinases (MEKKs). MEKKs typically act in conjunction with other proteins. For example, Raf-1 requires Ras and B-Raf 10 requires Rap1. Other proteins also influence MEKK activity such as heat shock protein 90, p50, and 14-3-3. Acting with these other proteins, the Rafs are the major class of proteins responsible for the activation of the MEKs, which are immediately upstream of ERK. There are 15 three forms of MEKs including MEK1a, MEK1b and MEK2 each of which specifically activates ERKs. The JNK family has a separate set of activating kinases known as SKK1/SEK1, which are activated independently of the Rafs.

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All of the ERKs are activated by dual phosphorylation on an activation loop that contains a threonine and tyrosine separated by a glutamate. The tyrosine is phosphorylated first. The ERKs with a single phosphorylation accumulate in the cell to a threshold 25 level, above which they are converted to the fully active, dual-phosphorylated form. After activation, an ERK translocates to the nucleus, where it modulates the activity of a number of transcription factors involved with the regulation of normal and aberrant cell growth, 30 including c-Myc, Elk-1 and ATF2. An ERK can also interact with other factors involved in DNA and protein

synthesis including, for example, other kinases, such as Rsk2, which phosphorylates histone H3; MAP Kinase interacting kinases (Mnk) 1 and 2, which are responsible for activating eukaryotic initiation factor 4E (eIF-4E), 5 which initiates protein synthesis; heat shock factor transcription factor 1 (hsp1); and topoisomerase II-b, among others. The JNKs activate transcription factors as well including, for example, c-Jun, Elk-1, Elk-2, ATF2 and serum response factor accessory protein (Sap-1). The 10 p38 kinases also activate ATF2 and Elk.

A method of the invention can be used to deliver an effective amount of electromagnetic energy to modulate a component of a mitogenic signal transduction 15 pathway set forth above. Modulation of the activity of a component in a signal transduction pathway can lead to changes in the activity of other components in the pathway or in a related pathway in accordance with the molecular interactions set forth above as well as others 20 known in the art as described, for example, in Houslay et al., Molecular Pharmacology 58:659-668 (2000); Lopez-Illasaca, Biochemical Pharmacology 56:269-277 (1998); Marinissen et al., Sciences 22:368-376 (2001); and Pearson et al., Endocrine Reviews 22:153-183 (2001).

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Changes in the activity or level of a cellular component can be correlated with other effects of delivery of electromagnetic energy such that changes in the activity or level of a cellular component can be 30 monitored to determine the effectiveness of electromagnetic therapy. Accordingly, the invention provides a method for monitoring progress of

electromagnetic therapy, by detecting a level of a cellular component in a cell population following delivery of electromagnetic energy to the cell population, whereby the level of the cellular component 5 correlates with the effectiveness of the therapy. The method can be used with any cellular component that changes activity or level in response to electromagnetic energy such as those set forth above and in the Examples.

10           The progress of electromagnetic therapy can be monitored based on the activity or level of a single gene product or a plurality of gene products. The level or activity of a gene product can be determined using methods well known in the art such as mRNA detection 15 methods and protein detection methods described, for example, in Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Press, Plainview, New York (1989); Sambrook et al., Molecular Cloning: A Laboratory Manual, 3rd ed., Cold Spring Harbor 20 Press, Plainview, New York (2001) or Ausubel et al. (Current Protocols in Molecular Biology (Supplement 47), John Wiley & Sons, New York (1999)). Additionally, activity of gene products can be measured using known enzyme assays such as kinase assays or binding assays 25 that exploit interactions and activities such as those described above in regard to particular gene products.

30           Monitoring a plurality of gene products provides the advantage of being able to determine the effects that the treatment has upon a signal transduction pathway or a network of interacting pathways. Examples

of methods known in the art for measuring the levels of a plurality of gene products include cDNA sequencing, clone hybridization, differential display, subtractive hybridization, cDNA fragment fingerprinting, serial analysis of gene expression (SAGE), and mRNA or protein microarrays. Example II describes the use of micro-array analysis for determining changes in expression for plurality of gene products in response to the delivery of electromagnetic energy. Methods of detecting the activity or level of one or more gene products can be performed either qualitatively or quantitatively.

Based on the activity or level of a cellular component determined in a diagnostic method of the invention, a course of therapy can be modified. In this regard, the invention provides a method for modifying electromagnetic therapy. The method includes the steps of: (a) detecting a level of a cellular component in a cell population following delivery of electromagnetic energy to the cell population, whereby the level of the cellular component correlates with the growth of the cell population, and (b) modifying the electromagnetic therapy based on the level of the cellular component in the cell population.

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One or more of the cellular components described herein can be detected in a method of modifying electromagnetic therapy. The effective dose of electromagnetic energy can be reduced or increased depending upon the particular cellular component detected and its level. In the case where a cellular component is detected to be above a desired level, the effective dose

of electromagnetic energy can be reduced. On the other hand, if a particular cellular component is detected to be below a desired level, the effective dose of electromagnetic therapy can be increased. The desired 5 level of one or more cellular components can be determined based on a correlation with desired outcomes in a model system or patient population in a clinical setting or using other correlation analyses known in the art. Electromagnetic therapy can be modified by altering 10 one or more of the parameters described above such that the effective amount of electromagnetic energy delivered to the cell or tissue being treated is either increased or decreased.

15 The molecular processes regulating the main events of the cell cycle are similar in all eucaryotic cells. Thus, an effective amount of electromagnetic energy when delivered to any eukaryotic cell in a method of the invention can be used to accelerate its cell 20 cycle. Examples of cells that are useful in a method of the invention are described below in the context of particular applications of the invention such as wound healing in which the cell cycle is accelerated for stromal cells, fibroblasts, keratinocytes, neutrophils, 25 epithelial cells or macrophages; healing of neuronal damage in which the cell cycle of neuronal cells and glia is accelerated; and production of artificial tissues in which the cell cycle is accelerated for fibroblasts, smooth muscle cells, endothelial cells, plasma cells, 30 mast cells, macrophages/monocytes, adipocytes, pericytes

reticular cells found in bone marrow stroma, or chondrocytes.

Electromagnetic energy can be delivered to a 5 cell *in vitro* or *in vivo* using a method of the invention. A cell that is treated *in vitro* using a method of the invention can be a primary cell or tissue sample obtained directly from an individual. A cell or tissue can be readily obtained using minimally invasive methods, for 10 example, from fluids such as the blood or lymph or from accessible tissues such as the skin, hair follicles, cervix or cheek. Where necessary a cell can also be obtained using slightly more invasive procedures, such as a punch biopsy, needle biopsy, endoscope biopsy or 15 surgical biopsy. Depending on the need and the availability of an appropriate procedure, cells from essentially any organ or tissue of the body can be obtained for use in a method of the invention. Those skilled in the art will know or be able to determine an 20 appropriate method for obtaining a cell of interest based on various factors including, for example, the location of the cell and risk factors or preference of the individual from whom the cell is harvested.

25 A cell used in an *in vitro* embodiment of the invention can be further isolated from other biological components. For example, a cell that is treated with electromagnetic energy can be a primary cell disaggregated from connective tissue and irrelevant cells 30 using, for example, known methods such as enzymatic digestion and biochemical separation. Likewise, a cell used in a method of the invention can be separated from

other cells, for example, using affinity separation methods known in the art. As an example, flow cytometry, selective media or antibody panning methods can be used to select a population of cells expressing a detectable 5 surface marker. Thus, a cell used in a method of the invention can be a single isolated cell or a cell in a population of cells such as a biological fluid, tissue or organ. A cell whether isolated or in a tissue or other population can be propagated in culture for several 10 generations, if desired. Cells can be propagated using methods known in the art as described, for example, in Freshney, Culture of Animal Cells, 4th Ed. Wiley-Liss, New York (2000).

15                   Delivery of electromagnetic energy to cells *in vitro* can be used to increase the rate at which the culture propagates. Thus, the methods can be used to decrease the cost and time required to obtain a cell culture that has grown to a desired density or to a point 20 of acquiring other favorable characteristics. A culture that has been stimulated to proliferate *in vitro* in a method of the invention can be used in an *in vitro* application. Examples of *in vitro* applications for which a cell treated in a method of the invention can be used 25 include diagnostic methods, cell based drug screening methods or biofermentation methods for production of biological agents. Alternatively, a cell or population of cells that has been stimulated to proliferate *in vitro* can be subsequently administered to an individual in an 30 *in vivo* therapeutic method. For example, the methods can be used to stimulate formation of a tissue *in vitro* under

conditions in which a replacement tissue or organ is formed. Once formed or grown to an appropriate stage, a tissue or organ can be administered to an individual in need of the tissue or organ. The use of electromagnetic 5 energy to stimulate tissue formation *in vitro* as well as *in vivo* is described in further detail below.

Electromagnetic energy can be delivered directly to a cell or to the environment of the cell such 10 as a culture medium, tissue, fluid or organ in which the cell is located. For *in vivo* applications of the method, electromagnetic radiation can be delivered directly to a site to be treated or to a location that is sufficiently proximal that the target cell will be electromagnetically 15 affected. As an example, electromagnetic energy can be delivered externally to treat conditions or diseases that afflict cells of the skin or that afflict internally located cells that are electromagnetically affected by surface application of electromagnetic energy. 20 Alternatively, electromagnetic energy can be delivered to an internal site by surgical exposure of the site or endoscopic access to the site.

A cell used in a method of the invention can be 25 genetically manipulated, for example, to include an exogenous nucleic acid. Thus, a method of the invention can include a step of introducing an exogenous nucleic acid into a cell to which electromagnetic energy is delivered. An exogenous nucleic acid can be introduced 30 into a cell using well known methods of transduction or transfection as described, for example, in Freshney et al., *supra* (2000); Sambrook et al., Molecular Cloning: A

Laboratory Manual, 2nd ed., Cold Spring Harbor Press, Plainview, New York (1989); Sambrook et al., Molecular Cloning: A Laboratory Manual, 3rd ed., Cold Spring Harbor Press, Plainview, New York (2001) or Ausubel et al.

5 (Current Protocols in Molecular Biology (Supplement 47), John Wiley & Sons, New York (1999)). An exogenous nucleic acid can be introduced into a cell in order to provide a diagnostic capability to the cell. Exemplary exogenous nucleic acids that can provide a diagnostic 10 capability to a cell include, without limitation, those that express reporter genes such as Green Fluorescent Protein (GFP), and wavelength shifted variants thereof; chloramphenicol acetyltransferase; beta-galactosidase; beta-glucuronidase; or luciferase. An exogenous nucleic 15 acid that is introduced into a cell can also express a therapeutic gene product such as a growth factor, hormone, or blood clotting factor. A therapeutic gene product can be expressed *in vitro* and subsequently delivered to an individual in need of the gene product in 20 a pharmaceutical formulation or a cell expressing a therapeutic gene product can be introduced into an individual in need of the gene product in order to treat a disease or condition. Similarly, an exogenous nucleic acid encoding other gene products that are useful in the 25 manufacture or production of therapeutics, foods, or industrial chemicals can be produced *in vitro* from a cell containing the nucleic acid. Electromagnetic energy can be delivered to a cell that contains an exogenous nucleic acid prior to expression of the nucleic acid or 30 concurrently with its expression.

The invention also provides a method for reducing inflammation. The method includes a step of delivering to a tissue undergoing inflammation an effective amount of electromagnetic energy to reduce the 5 inflammation. A collection of immune system cells and molecules at a target site is known in the art as inflammation, a common response to injury or infection that is identified by four classic symptoms including heat (calor), redness (rubor), swelling (tumor) and pain 10 (dolor). Acute inflammatory response, which is induced by antibodies or other agents, is characterized by a set of rapidly occurring events at the site of injury. Vessels located near the site of the injury dilate, thereby causing redness and heat, allowing an influx of 15 plasma proteins and phagocytic cells into the tissue spaces, thereby causing swelling. Release and/or activation of other inflammation mediators, and increased tissue pressure, stimulate local nerve endings, causing pain. The methods of the invention can be used to reduce 20 or ameliorate symptoms associated with inflammation. Delivery of electromagnetic energy in the methods leads to reduction in inflammation by promoting reduction in inflammatory processes occurring in the cells set forth below, thereby allowing progression to subsequent healing 25 stages.

In an infection, if the acute inflammatory response relieves the host of the infectious agent, repair and regeneration ensue. However, if the acute 30 response is not effective in ridding the host of the infection, the continued influx of polymorphonuclear leukocytes and serum products can lead to formation of

abscesses and granulomas. The abscess is a swelling which is bounded by fibrin from clotted blood and cells involved in phagocytosis and repair. The central cavity of the abscess contains both live and dead 5 polymorphonuclear leukocytes, tissue debris, and the remaining injurious or infecting agent.

A continuing acute inflammatory response can lead to a chronic inflammatory response, which is 10 associated with the same four clinical signs described above, but is composed of additional cellular and soluble mediators. Chronic inflammatory responses are characterized by an infiltration of lymphocytes and cells of monocyte-macrophage lineage in addition to 15 polymorphonuclear cells.

Both acute and chronic inflammation include three phases. In the first phase, the material to be eliminated (antigen) is recognized as foreign by various 20 mechanisms involving immunoglobulins. Following recognition, the second phase of the immune response is initiated, during which an amplification system involving complement, cytokines, kinins, coagulation, lipid mediators, and a large number of inflammatory cells is 25 activated. This results in an alteration of blood flow, increased vascular permeability, augmented adherence of circulating leukocytes to the vascular endothelium, promotion of migration of leukocytes into tissue, and stimulation of leukocytes to destroy the inciting 30 antigen. During the third phase, destruction of the antigen is mediated by several non-specific mechanisms

including phagocytic cells such as neutrophils, eosinophils and mononuclear phagocytes. Such phagocytic leukocytes migrate freely or are fixed in tissue sites as components of the mononuclear phagocyte system.

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The first immune cells to arrive at the site of inflammation are neutrophils, generally within a few hours of tissue injury or infection. Neutrophils are produced in the bone marrow and take approximately two weeks to achieve maturity. The first seven days of neutrophil development are proliferative, and with successive cell division the cells evolve from myeloblasts to promyelocytes and then to myelocytes. During this period neutrophils acquire their characteristic granules. The first granules to appear during neutrophil maturation are called the primary or azurophil granules. Primary granules function predominantly in the intercellular environment, in the phagolysosomal vacuole where they are involved in killing and degrading microorganisms.

Macrophages perform a similar function to neutrophils as well as more diverse tasks. These ubiquitous and mobile cells continually sample their environment and respond to various stimuli. Macrophages are highly active in absorptive endocytosis or pinocytosis, and in receptor-mediated endocytosis. When particles are internalized by these processes, antimicrobial and general cytotoxic activity is promoted, thereby killing infectious agents.

Wound repair is an example of a healing process that is characterized by an initial inflammatory response followed by later stages of healing. In particular, the natural course of wound healing to closure occurs in four phases identified as acute inflammation, granulation, epithelialization and tissue remodeling. During the inflammation phase there is an immigration of neutrophils into the area of injury within the first 24 hours. Within the subsequent 24 to 48 hours, the immunocyte profile changes as the infiltrate begins to consist predominantly of macrophages and lymphocytes. In another 24 to 48 hours, macrophages and lymphocytes become the predominant cell types within wound tissue. It is also during the inflammatory phase that monocytes are converted to macrophages, which release growth factors for stimulating angiogenesis and the production of fibroblasts. In one embodiment of the invention, wound repair is accelerated by delivering to the wound an effective amount of electromagnetic energy to reduce inflammation at the wound.

In further embodiments the methods of the invention can be used to deliver electromagnetic energy to other tissues undergoing inflammation to reduce the inflammation and promote healing. Examples of tissues that can be treated in a method of the invention when undergoing inflammation include, without limitation, neural tissue associated with a neuroinflammatory disorder, gastrointestinal tissue associated with an inflammatory bowel disorder or ulcer, synovium tissue associated with arthritic inflammation, lung tissue

associated with asthma, or skin associated with an inflammatory skin condition such as psoriasis, eczema or atopic dermatitis. The cell or tissue to which electromagnetic energy is delivered in a method of the 5 invention can be one that is not associated with a wound. Thus, although the methods are exemplified herein with respect to wounds, the methods can be used to treat inflammation associated with a disease or condition other than a wound.

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As described in further detail below, the methods of the invention are useful for reducing both acute and chronic inflammation. For example, the methods of the invention are useful for reducing acute 15 inflammation associated with, for example, swelling resulting from bumps (contusions), bruises, sprains, abrasions, cuts, insect stings, plant-induced contact dermatitis as can be caused by plants such as poison ivy, poison oak or poison sumac.

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The methods of the invention also are useful for reducing the severity of a neuroinflammatory disorder, for example, a demyelinating disease. A central mechanism in the pathology of neuroinflammatory 25 demyelinating diseases is the organ-specific migration of activated T lymphocytes into the brain. Additionally, injury to the spinal cord precipitates the activation of resident microglia and the recruitment of circulating inflammatory cells, including macrophages and 30 lymphocytes. These cells can cause tissue damage and loss of neurological function via autoimmune reactions to myelin proteins. Autoimmunity can be trauma-induced

leading to ongoing central nervous system (CNS) immunologic responses by the autoreactive repertoire. Accordingly, the methods of the invention are applicable in the context of CNS trauma and neurodegenerative 5 diseases such as for example, Multiple Sclerosis (MS), Chronic Inflammatory Demyelinating Polyneuropathy, Amyotrophic Lateral Sclerosis (ALS) and Alzheimer's Disease.

10 Demyelinating diseases are an important group of neurological disorders because of the frequency with which they occur and the disability that they cause. Demyelinating diseases have in common a focal or patchy destruction of myelin sheaths that is accompanied by a 15 neuroinflammatory response. Neuroinflammatory demyelinating diseases can be divided into processes affecting myelin of the central nervous system and those affecting myelin of the peripheral nervous system. Multiple Sclerosis (MS) is a central nervous system 20 demyelinating disease with an autoimmune etiology as reviewed in Martin et al., Annu. Rev. Immunol. 10:153-187 (1992). Other demyelinating diseases of the central nervous system include, for example, acute disseminated encephalomyelitis (ADE) including postinfectious and 25 postvaccinal encephalomyelitis, acute necrotizing hemorrhagic encephalomyelitis and progressive (necrotizing) myelopathy. Demyelinating diseases of the peripheral nervous system include, for example, acute inflammatory demyelinating polyradiculoneuropathy 30 (Guillain-Barré syndrome), chronic inflammatory demyelinating polyradiculoneuropathy (CIDP),

demyelinating neuropathy associated with IgM monoclonal gammopathy and neuropathy associated with sclerosing myeloma. A method of the invention can be used to treat a neuroinflammatory disease or condition by delivering 5 to a neural tissue undergoing inflammation an effective amount of electromagnetic energy to reduce the inflammation.

The methods of the present invention are 10 further useful for reducing inflammation associated with Crohn's disease (CD) and ulcerative colitis (UC), two gastrointestinal disorders that are collectively referred to as Inflammatory Bowel Disease (IBD); or regional enteritis, which is a disease of chronic inflammation 15 that can involve any part of the gastrointestinal tract, by delivering an effective amount of electromagnetic energy to a cell or tissue at a site undergoing inflammation associated with these disorders. Commonly the distal portion of the small intestine (ileum) and 20 cecum are affected. In other cases, the disease is confined to the small intestine, colon or anorectal region. Crohn's disease occasionally involves the duodenum and stomach, and more rarely the esophagus and oral cavity.

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Several features are characteristic of the pathology of Crohn's disease. The inflammation associated with CD, known as transmural inflammation, involves all layers of the bowel wall. Thickening and 30 edema, for example, typically appear throughout the bowel wall, with fibrosis also present in long-standing disease. The inflammation characteristic of CD also is

discontinuous with segments of inflamed tissue, known as "skip lesions," separated by apparently normal intestine. Furthermore, linear ulcerations, edema, and inflammation of the intervening tissue lead to a "cobblestone" 5 appearance of the intestinal mucosa, which is distinctive of CD.

A hallmark of Crohn's disease is the presence of discrete aggregations of inflammatory cells, known as 10 granulomas, which are generally found in the submucosa. About half of Crohn's disease cases display the typical discrete granulomas, while others show a diffuse granulomatous reaction or nonspecific transmural 15 inflammation. As a result, the presence of discrete granulomas is indicative of CD, although the absence granulomas also is consistent with the disease. Thus, transmural or discontinuous inflammation, rather than the presence of granulomas, is a preferred diagnostic 20 indicator of Crohn's disease (Rubin and Farber, Pathology (Second Edition) Philadelphia: J.B. Lippincott Company (1994)).

The methods of the present invention are also useful for reducing inflammation associated with 25 ulcerative colitis by delivering an effective amount of electromagnetic energy to a cell or tissue at a site affected by UC. Several pathologic features characterize UC in distinction to other inflammatory bowel diseases. Ulcerative colitis is a diffuse disease that usually 30 extends from the most distal part of the rectum for a

variable distance proximally. The term left-sided colitis describes an inflammation that involves the distal portion of the colon, extending as far as the splenic flexure. Sparing of the rectum or involvement of the right side (proximal portion) of the colon alone is unusual in ulcerative colitis. Furthermore, the inflammatory process of UC is limited to the colon and does not involve, for example, the small intestine, stomach or esophagus. In addition, ulcerative colitis is distinguished by a superficial inflammation of the mucosa that generally spares the deeper layers of the bowel wall. Crypt abscesses, in which degenerate intestinal crypts are filled with neutrophils, also are typical of the pathology of ulcerative colitis (Rubin and Farber, 1994, Pathology (Second Edition) Philadelphia: J.B. Lippincott Company (1994), which is incorporated herein by reference).

A characteristic endoscopic feature of UC, which when present with clinical features of left-sided colonic disease indicates ulcerative colitis, is inflammation that is more severe distally than proximally or continuous inflammation. Additional typical endoscopic features that may be present in UC include inflammation extending proximally from the rectum or shallow ulcerations or the lack of deep ulcerations.

A method of the invention can also be useful for reducing inflammation occurring at a joint, for example, associated with arthritis. An example of a joint disease is rheumatoid arthritis (RA) which involves

inflammatory changes in the synovial membranes and articular structures as well as muscle atrophy and rarefaction of the bones, most commonly the small joints of the hands. Inflammation and thickening of the joint 5 lining, called the synovium, can cause pain, stiffness, swelling, warmth, and redness. The affected joint may also lose its shape, resulting in loss of normal movement and, if uncontrolled, may cause destruction of the bones, deformity and, eventually, disability. In some 10 individuals, RA can also affect other parts of the body, including the blood, lungs, skin and heart. A method of the invention can be useful for reducing one or more of these adverse symptoms by reducing inflammation associated with RA.

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A method of the invention can be used to replace damaged tissue by treating the damaged tissue with an effective amount of electromagnetic energy to stimulate growth of a replacement tissue. Examples of 20 tissues that can be replaced in a method of the invention include, without limitation, epithelial tissue, bone marrow tissue, smooth muscle tissue, connective tissue, adrenal tissue and neurological tissue. A tissue that is replaced in a method of the invention can be located 25 anywhere in the body that is accessible to delivery of electromagnetic energy including, for example, in a blood vessel, vein, artery, tendon, ligament, gastrointestinal tract, genitourinary tract, bone marrow, skin, liver, pancreas, lung, kidney, or nervous system including the 30 central or peripheral nervous system. Because the inability to restore and preserve normal tissue structure

after damage is a major cause of organ failure, such as failure of the liver, kidney, or heart, the methods of the invention are particularly useful for reducing the risk of organ failure.

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A replacement tissue that is induced to proliferate by delivery of electromagnetic energy in a method of the invention can be derived from native cells that are naturally occurring in the individual being treated. Examples of cells that are capable of replacing skin cells include those described above with respect to wound healing. Cells that can be stimulated in a method of the invention to replace a tissue of the circulatory system include, for example, fibroblasts, smooth muscle cells, and endothelial cells. The methods can be used to simultaneously stimulate proliferation of distinct cell types such as fibroblasts, smooth muscle cells, capillary cells or lymphocytes that are useful for replacing tissues of the gastrointestinal tract. Stimulation of fibroblasts is also useful for replacing tendons and ligaments. Parenchymal cells and other known tissue specific cells can be used to replace damaged portions of organs. Damaged tissue of the nervous system can be replaced with neurons or glia cells. It will be understood that replacement of the cell types set forth above by stimulating their proliferation includes stimulation of precursor cells and stem cells that differentiate into the cell types set forth above.

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The invention further provides a method for stimulating growth of administered cells. The method includes the steps of (a) administering a population of

cells to an individual, and (b) delivering to the population an effective amount of electromagnetic energy to stimulate growth of the population. In one embodiment, the population of cells can be administered to a site of tissue damage, such as those described above, and stimulated to replace the damaged tissue. A population of cells can also be administered in a method of treating other defects in the body such as the deficiency or over abundance of a particular gene product. Accordingly, a method of the invention can include administering cells that either naturally express an effective amount of a gene product for a desired therapeutic effect or that have been genetically manipulated to do so using, for example, the methods described above.

A cell or population of cells administered to an individual in a method of the invention can be any type of cell that is appropriate for replacing a tissue or performing a desired function including, for example, those set forth above. A population of cells that is administered in a method of the invention can be in a tissue or organ that is isolated as a tissue or organ from a donor individual or that is produced in a culture system. Methods for producing synthetic tissues or organs are set forth in further detail below.

For therapeutic applications, the above cell types are additionally chosen to remain viable *in vivo* without being substantially rejected by the host immune system. Therefore, the donor origin of the cell type

should be evaluated when selecting cells for therapeutic administration. A cell can be autologous, wherein it is administered to the same individual from whom it was removed or can be heterologous being obtained from a 5 donor individual who is different from the recipient individual. Those skilled in the art know what characteristics should be exhibited by cells to remain viable following administration. Moreover, methods well known in the art are available to augment the viability 10 of cells following administration into a recipient individual.

One characteristic of a donor cell type substantial immunological compatibility with the 15 recipient individual. A cell is immunologically compatible if it is either histocompatible with recipient host antigens or if it exhibits sufficient similarity in cell surface antigens so as not to elicit an effective host anti-graft immune response. Specific examples of 20 immunologically compatible cells include autologous cells isolated from the recipient individual and allogeneic cells which have substantially matched major histocompatibility (MHC) or transplantation antigens with the recipient individual. Immunological compatibility 25 can be determined by antigen typing using methods well known in the art. Using such methods, those skilled in the art will know or can determine what level of antigen similarity is necessary for a cell or cell population to be immunologically compatible with a recipient 30 individual. Tolerable differences between a donor cell and a recipient can vary with different tissues and can be readily determined by those skilled in the art.

In addition to selecting cells which exhibit characteristics that maintain viability following administration to a recipient individual, methods well known in the art can be used to reduce the severity of immunorejection. Such methods can be used to further increase the *in vivo* viability of immunologically compatible cells or to allow the *in vivo* viability of less than perfectly matched cells or of non-immunologically compatible cells. Therefore, for therapeutic applications, it is not necessary to select a cell type from the recipient individual to achieve viability of the modified cell following administration. Instead, and as described further below, alternative methods can be employed which can be used in conjunction with essentially any donor cell to confer sufficient viability of the modified cells to achieve a particular therapeutic effect.

For example, in the case of partially matched or non-matched cells, immunosuppressive agents can be administered to render the host immune system tolerable to administration of the cells. The regimen and type of immunosuppressive agent to be administered will depend on the degree of MHC similarity between the donor cell and the recipient. Those skilled in the art know, or can determine, what level of histocompatibility between donor and recipient antigens is applicable for use with one or more immunosuppressive agents. Following standard clinical protocols, administration and dosing of such immunosuppressive agents can be adjusted to improve

viability of the cells *in vivo*. Specific examples of immunosuppressive agents useful for reducing a host immune response include, for example, cyclosporin, corticosteroids, and the immunosuppressive antibody known 5 in the art as OKT3.

Another method which can be used to confer sufficient viability of partially-matched or non-matched cells is through the masking of the cells or of one or 10 more MHC antigen(s) to protect the cells from host immune surveillance. Such methods allow the use of non-autologous cells in an individual. Methods for masking cells or MHC molecules are well known in the art and include, for example, physically protecting or concealing 15 the cells, as well as disguising them, from host immune surveillance. Physically protecting the cells can be achieved, for example, by encapsulating the cells within a barrier device. Alternatively, antigens can be disguised by treating them with binding molecules such as 20 antibodies that mask surface antigens and prevent recognition by the immune system.

Immunologically naive cells also can be administered in a method of the invention.

25 Immunologically naive cells are devoid of MHC antigens that are recognized by a host immune system. Alternatively, such cells can contain one or more antigens in a non-recognizable form or can contain modified antigens that mirror a broad spectrum of MHC 30 antigens and are therefore recognized as self-antigens by most MHC molecules. The use of immunologically naive cells therefore has the added advantage of circumventing

the use of the above-described immunosuppressive methods for augmenting or conferring immunocompatibility onto partially or non-matched cells. As with autologous or allogeneic cells, such immunosuppressive methods can 5 nevertheless be used in conjunction with immunologically naive cells to facilitate viability of the administered cells.

An immunologically naive cell, or broad 10 spectrum donor cell, can be obtained from a variety of undifferentiated tissue sources, as well as from immunologically privileged tissues. Undifferentiated tissue sources include, for example, cells obtained from embryonic and fetal tissues. An additional source of 15 immunologically naive cells include stem cells and lineage-specific progenitor cells. These cells are capable of further differentiation to give rise to multiple different cell types. Stem cells can be obtained from embryonic, fetal and adult tissues using 20 methods well known to those skilled in the art. Such cells can be used directly or modified further to enhance their donor spectrum of activity.

Immunologically privileged tissue sources 25 include those tissues which express, for example, alternative MHC antigens or immunosuppressive molecules. A specific example of alternative MHC antigens are those expressed by placental cells, which prevent maternal anti-fetal immune responses. Additionally, placental 30 cells are also known to express local immuno-suppressive

molecules that inhibit the activity of maternal immune cells.

An immunologically naive cell or other donor cell can be modified to express genes encoding, for example, alternative MHC or immuno-suppressive molecules that confer immune evasive characteristics. Such a broad spectrum donor cell, or similarly, any of the donor cells described previously, can be tested for immunological compatibility by determining its immunogenicity in the presence of recipient immune cells. Methods for determining immunogenicity and criteria for compatibility are well known in the art and include, for example, a mixed lymphocyte reaction, a chromium release assay or a natural killer cell assay. Immunogenicity can be assessed by culturing donor cells together with lymphocyte effector cells obtained from a recipient individual and measuring the survival of the donor cell targets. The extent of survival of the donor cells is indicative of, and correlates with, the viability of the cells following implantation.

The invention further provides a method for stimulating formation of a tissue. The method includes the steps of: (a) contacting a population of cells with a matrix under conditions suitable for tissue formation by the cells, and (b) delivering to the population an effective amount of electromagnetic energy to stimulate formation of the tissue. One or both of the steps of the method can be carried out either *in vitro* or *in vivo*.

A matrix can be used in a method of the invention to provide a structural scaffold for a tissue. Such a scaffold can provide a substrate to which a tissue is adhered thereby localizing the tissue to a particular 5 location in the body. If desired, the matrix can further be shaped to produce a tissue with a desired morphology. Examples of materials that are particularly useful as 10 matrices include, without limitation, nylon (polyamides), dacron (polyesters), polystyrene, polypropylene, polyacrylates, polyvinyl compounds (e.g., 15 polyvinylchloride), polycarbonate (PVC), polytetrafluorethylene (PTFE; teflon), thermanox (TPX), nitrocellulose, polyglycolic acid (PGA), cotton, cat gut sutures, cellulose, gelatin, dextran or an *in vivo* site such as bone, other tissues or a wound.

A matrix can be pre-treated prior to inoculation of cells in order to enhance the attachment of the cells to the matrix. For example, prior to 20 inoculation with cells, nylon matrices can be treated with 0.1M acetic acid, and incubated in polylysine, FBS, and/or collagen to coat the nylon. Polystyrene can be similarly treated using sulfuric acid.

25 Where the matrix and cells are to be implanted *in vivo*, it may be preferable to use biodegradable matrices such as polyglycolic acid, catgut suture material, or gelatin, for example. Where the cultures are to be maintained for long periods of time or 30 cryopreserved, non-degradable materials such as nylon, dacron, polystyrene, polyacrylates, polyvinyls, teflons

or cotton can be used. A convenient nylon mesh that can be used in accordance with the invention is Nitex, a nylon filtration mesh having an average pore size of 210 microns and an average nylon fiber diameter of 90 microns  
5 (#3-210/36, Tetko, Inc., N.Y.).

Conditions suitable for *in vivo* formation of a tissue when a population of cells is contacted with a matrix include those described above in regard to  
10 replacement of damaged tissues except that the matrix is provided in a manner that does not interfere with the process of tissue replacement. In general, the matrix is provided under sterile conditions to avoid infection of the damaged tissue site. The matrix is further disposed  
15 in an orientation that allows cells to adhere to the matrix and, if desired, migrate along the matrix to form the tissue. The methods described above for delivering electromagnetic energy to a population of cells for replacing damaged tissue can be used in the presence of a  
20 matrix as well.

Conditions suitable for *in vitro* formation of a tissue when a population of cells is contacted with a matrix are known in the art and described, for example,  
25 in U.S. Pat Nos. 5,842,477; 5,863,531; 5,902,741 and 5,962,325. An *in vitro* tissue can be cultured in a closed system bioreactor as described, for example, in U.S. Pat No. 6,121,042. The methods and apparatus known in the art can be modified to deliver electromagnetic  
30 energy to a cell culture system. Those skilled in the art will be able to make such modifications by providing

an electromagnetic energy delivery device to the culture system according to the teachings described herein.

A method of the invention for stimulating formation of a tissue *in vitro* can further include a step of administering the tissue to an individual. Such a tissue can be administered using methods described above with regard to administering cells to wounds and other sites of tissue damage. Those skilled in the art will know or be able to determine placement of a synthetic tissue based on structural properties inherent in the tissue such as morphology and cell composition. Cell types and tissues capable of the *in vivo* or *in vitro* formation methods of the invention include, for example, 15 macrophages, neutrophils, fibroblasts, muscle cells, epithelial cells, keratinocytes, microvascular and other endothelial cells, epidermal melanocytes, hair follicle papilla cells, skeletal muscle cells, smooth muscle cells, osteoblasts, neurons, chondrocytes, hepatocytes, 20 pancreatic cells, kidney cells, aortic cells, bronchial/tracheal cells (both epithelial and muscle cells).

The following examples are intended to 25 illustrate but not limit the present invention.

EXAMPLE I

5                   **Induction of Cell Proliferation with Electromagnetic  
Energy**

This example demonstrates delivery of electromagnetic energy to cells in vitro and activation of extracellular signal-regulated protein kinase 1 (ERK-1 10 or p44 kinase) and other components associated with mitogenic signaling pathways.

Primary Human Dermal Fibroblasts (HDF) and Human Epidermal Keratinocytes (HEK) (Cell Applications, 15 Inc., San Diego CA) were used between passages 3 and 15 and 3-8, respectively. Unless stated otherwise, all cell culture supplies were purchased from Mediatech Inc. (Herndon, VA). Minimum Essential Medium (MEM) was used for culture of the HDFs. This medium was supplemented 20 with 5% fetal bovine serum (Hyclone, Logan, UT), 1 mM sodium pyruvate, 100 U/ml penicillin G, 100 U/ml streptomycin and 1% non-essential amino acids. Serum-free growth media (Cell Applications, Inc. San Diego CA) was used for culturing the HEK cells.

25

The primary HDF cells were synchronized, then treated with radio frequency energy (RF) as follows. Briefly, cells plated at 1000 cells/well in 96 well plates were synchronized using Compactin as described in 30 Keyomarsi et al., Cancer Res. 51:3602-3609 (1991) with the following modifications. Media was removed 30-36 hours after and the cells were then treated with a 100X

excess of Mevalonolactone (MAL) to release them from Compactin synchronization. 30 minutes after MAL treatment the cells were treated with a 30 minute dose of RF at 32 mw/cm<sup>2</sup>.

5

As shown in Figure 1, treatment of HDF cells with RF induced proliferation. Furthermore, untreated cells showed induced proliferation when media from the RF treated cells was added within 1 hour. For the media transfer analysis, HDF cells in 5% FBS-MEM were plated at 10 2.5x10<sup>3</sup> cells/well in 96 well plates and allowed to attach overnight. Plates were treated with RF consisting of a dose of 32 mw/cm<sup>2</sup> provided as a train of 42  $\mu$ sec pulses delivered at a rate of 1Khz for 30 minutes. Media 15 was then removed after 1 hour, 3 hours, 6 hours, 16 hours, 24 hours after RF treatment and placed on untreated cells. All plates were assayed for cell growth twenty-four hours after RF treatment. Cell growth was quantified using CyQUANT™ Cell Proliferation Assay Kit 20 (Molecular Probes, Eugene, OR). Comparison of the stimulation of cell growth by RF treatment was similar to treatment of cells with known growth factors such as platelet derived growth factor (PDGF), further indicating that RF treatment of cells stimulates cell growth in a 25 similar fashion as endogenous growth factors.

In addition to inducing proliferation in the HDF cells, RF treatment induced the concomitant activation of the ERK signaling pathway. Levels of ERK 30 were detected as follows. Treated cells were washed once with cold phosphate-buffered saline, lysed in Laemmli

sample buffer (Bio-Rad, Hercules, CA) and sonicated. Samples were heated to 95°C, electrophoresed on 12.5% SDS gels and transferred to PVDF membrane (Osmonics, Inc., Westborough, MA) by semi-dry transfer in CAPS buffer (pH 5 11). The PVDF membrane was blocked in Tris-buffered saline (20 mM Tris-HCl, 130 mM NaCl, pH 7.6) containing 5% non-fat dry milk. The membrane was then incubated with anti-phosphorylated p44/42 MAP Kinase (Thr202/Tyr204) antibody or anti-p44/42 antibody (Cell 10 Signaling Technology, Beverly, MA) overnight at 4°C, and washed in Tris-buffered saline supplemented with 1% Tween-20 (AP Biotech, Piscataway, NJ). The membrane was visualized with the Amersham ECF kit (Piscataway, NJ) according to the manufacturers protocol and imaged on a 15 Storm 840 PhosphoImager.

Using the above-described Western blotting techniques, cell lysates were probed for the presence of activated ERK-1 and ERK-2 at specific times after 20 initiation of treatment. As shown in Figure 2, significant activation of ERK-1 and ERK-2, resulting in 250% increased levels compared to control cells occurred after the initiation of treatment. ERK activation within the first 30 minutes of treatment indicated that RF 25 activated this Kinase cascade in a biologically relevant time frame for affecting cellular functions such as cell cycle progression and cell proliferation.

To determine if RF treatment increased the rate 30 of entry into the S phase of the cell cycle, DNA synthesis was imaged in the synchronized HDF cells following treatment with RF. Entry into the S phase of

the cell cycle was determined with the Roche (Mannheim, Germany) BrdU labeling and detection kit. Briefly, cultured cells were labeled with BrdU, fixed with ethanol, and incubated with nucleases to partially digest 5 cellular DNA. Anti-BrdU antibody conjugated to peroxidase was incubated with the cells. Peroxidase substrate was added to the plates producing a colored product that was measured with an ELISA reader. As shown in Figure 3, the RF treated cells entered the S phase of 10 the cell cycle on average, 8 hours before untreated controls.

These results demonstrate that RF acts as an exogenous, non-molecular mitogen. The results further 15 demonstrate that RF induces the release of soluble factors via a transduction pathway that includes ERK-1, and that the resulting soluble factor release re-stimulates the mitogenic signaling pathway as demonstrated by the second phase of ERK-1 activation.

20

#### EXAMPLE II

#### **Activation of Molecular Regulatory Networks with 25 Electromagnetic Energy**

This example demonstrates delivery of electromagnetic energy to cells leading to modulation in the levels of gene products associated with molecular 30 regulatory networks. The levels of various components are shown to be modulated within the first few minutes to

several hours following delivery of electromagnetic energy.

HDF cells were cultured in MEM supplemented  
5 with 5% fetal calf serum as described in Example I. Cells were plated in 10 cm plates at a density of  $5 \times 10^5$  cells per plate. Twenty hours after plating electromagnetic energy was delivered to the cells as described in Example I. RNA was harvested from cells at  
10 various times according to the method of Chomczynski, P. and Sacchi, Analytical Biochemistry 162 pg. 156-159 (1987). Fifty  $\mu$ g of total RNA was treated with DNase I for 30 minutes followed by phenol extraction and ethanol precipitation. The RNA was then labeled with  $^{32}$ P dATP  
15 using reverse transcriptase. Labeled probes were then purified by column chromatography and then hybridized to micro-arrays at  $1 \times 10^5$  cpm/ml at 68°C for 24 hours. Micro-arrays were purchased from BD Biosciences Clontech (Palo Alto, CA). Two arrays were used: the Atlas array  
20 1.2 (1,174 cDNA clones) and a Stress array which contained 234 cDNA clones of genes related to cellular stress. The list of the genes and their sequences can be found on the world wide web at the website for BD Biosciences Clontech. The blots were washed and then  
25 exposed for 5-7 days at -80° C using double intensifying screens. Quantitation of transcript levels between blots was performed by normalization to housekeeping gene expression.

30 Time-dependent quantitation of the levels of gene expression in HDF cells and HEK cells is shown in Figures 6 and 7, respectively. The data was analyzed

using a K-means test for 5 expression groups (Clusters A-E), corresponding to early to late expression. In Figures 6A (HDF cells) and 7A (HEK cells), line graphs show the time-dependent expression profile for each 5 cluster of genes. Shown in Figures 6B-D and 7B-D are the expression profiles of the following functional families: Adhesion Molecules; Cyclins; DNA Synthesis Proteins; Growth Factors and corresponding Receptors; Interleukins; Interferons and corresponding Receptors; MAP Kinases; 10 other Kinases; Matrix Metalloproteinases and their Inhibitors; Protein Kinase Cs; Tumor Necrosis Factors and their Receptors; and Transcription Factors. In Figures 6B-D (HDF cells) and 7B-D (HEK cells) genes within 15 functional groups are listed from top to bottom based on onset of expression with early expressed genes at the top of each figure and progressively later expressed genes towards the bottom. From these findings it is clear that treatment of HDF cells with the RF field induces a large number of the genes studied in a programmed manner. The 20 genes that showed the earliest response included genes that encode extracellular matrix proteins and signal transduction. Genes involved in regulation of the cell cycle and DNA synthesis were transcribed at later time points, corresponding to the influx of signal from the 25 extracellular membrane through the cytoplasm, and into the nucleus.

Figure 5 shows autoradiographs from studies using the 234 gene micro-array related to inflammation 30 response. Delivery of electromagnetic energy induced a substantial number of genes to be expressed at levels

substantially greater than control. Examples of gene products that showed no response to electromagnetic energy and gene products that were induced by electromagnetic energy are indicated by the green and red 5 arrows, respectively.

Figure 4 shows autoradiographs from studies using the 1,176 gene micro-array related to the cell cycle and cell growth. As was found with the 234 gene 10 micro-array, the levels of many gene products were changed after delivery of electromagnetic energy. Table 1 below sets forth the names and corresponding Genbank Accession numbers for all genes that were found to have a significant (four-fold or more) increase in 15 expression following delivery of electromagnetic energy.

As described above, the types of gene products that are modulated by delivery of electromagnetic radiation can be classified into a number of groups 20 including extracellular matrix receptors, signal transduction proteins, cell cycle regulators, transcription factors and DNA synthesis proteins. The results summarized in Figures 6 and 7 demonstrate that electromagnetic energy modulates the activity of 25 molecular regulatory networks that mediate a number of inflammatory and cell proliferation responses such as wound healing.

Table 1. Genes showing at least 4-Fold Expression Increase upon Treatment with Electromagnetic Energy

Genbank Accession Number	Name
U18087	3'5'-cAMP phosphodiesterase HPDE4A6
U22456	5'-AMP-activated protein kinase catalytic alpha-1 subunit; AMPK alpha-1 chain
U41766	a disintegrin and metalloproteinase domain 9 (meltrin gamma)
L13738	activated p21cdc42Hs kinase
U12979	activated RNA polymerase II transcription cofactor 4
U14722	activin A receptor, type IB
M74088	adenomatosis polyposis coli
X68486	adenosine A2a receptor
X76981	adenosine A3 receptor
X74210	adenylate cyclase 2 (brain)
D25538	adenylate cyclase 7
AF036927	adenylate cyclase 9
M36340	ADP-ribosylation factor 1
M15169	adrenergic, beta-2-, receptor, surface
AB010575	amiloride-sensitive cation channel 3, testis
M20132	androgen receptor (dihydrotestosterone receptor; testicular feminization; spinal and bulbar muscular atrophy; Kennedy disease)
M87290	angiotensin receptor 1
M12154	antigen p97 (melanoma associated) identified by monoclonal antibodies 133.2 and 96.5
AF013263	apoptotic protease activating factor
U11700	ATPase, Cu++ transporting, beta polypeptide (Wilson disease)
U51478	ATPase, Na+/K+ transporting, beta 3 polypeptide
U45878	baculoviral IAP repeat-containing 3
M14745	B-cell CLL/lymphoma 2
M31732	B-cell CLL/lymphoma 3
U00115	B-cell CLL/lymphoma 6 (zinc finger protein 51)
U15172	BCL2/adenovirus E1B 19kD-interacting protein 1
U66879	BCL2-antagonist of cell death
L22474	BCL2-associated X protein
X89986	BCL2-interacting killer (apoptosis-inducing)
Z23115	BCL2-like 1
U59747	BCL2-like 2
U29680	BCL2-related protein A1
D21878	bone marrow stromal cell antigen 1
M22491	bone morphogenetic protein 3 (osteogenic)
M60315	bone morphogenetic protein 6
M97016	bone morphogenetic protein 8 (osteogenic protein 2)
U76638	BRCA1 associated RING domain 1

Genbank Accession Number	Name
X58957	Bruton agammaglobulinemia tyrosine kinase
AF046079	budding uninhibited by benzimidazoles 1 (yeast homolog), beta
Z13009	cadherin 1, type 1, E-cadherin (epithelial)
X79981	cadherin 5, type 2, VE-cadherin (vascular epithelium)
L00587	calcitonin receptor
M94172	calcium channel, voltage-dependent, L type, alpha 1B subunit
L41816	calcium/calmodulin-dependent protein kinase I
L24959	calcium/calmodulin-dependent protein kinase IV
M23254	calpain 2, (m/II) large subunit
X04106	calpain, small subunit 1
M31630	cAMP response element binding protein (CRE-BP1); transcription factor ATF2; HB16
L05515	cAMP response element-binding protein CRE-BP $\alpha$
U89896	casein kinase 1, gamma 2
J02853	casein kinase 2, alpha 1 polypeptide
U84388	CASP2 and RIPK1 domain containing adaptor with death domain
U13699	caspase 1, apoptosis-related cysteine protease (interleukin 1, beta, convertase)
U13737	caspase 3, apoptosis-related cysteine protease
X87838	catenin (cadherin-associated protein), beta 1 (88kD)
M11233	cathepsin D (lysosomal aspartyl protease)
X12451	cathepsin L
M37197	CCAAT-box-binding transcription factor
L25259	CD86 antigen (CD28 antigen ligand 2, B7-2 antigen)
U05340	CDC20 (cell division cycle 20, <i>S. cerevisiae</i> , homolog)
X54941	CDC28 protein kinase 1
X54942	CDC28 protein kinase 2
L29222	CDC-like kinase 1
U03882	chemokine (C-C motif) receptor 2
X91906	chloride channel 5 (nephrolithiasis 2, X-linked, Dent disease)
M30185	cholesteryl ester transfer protein, plasma
U62439	cholinergic receptor, nicotinic, beta polypeptide 4
U33286	chromosome segregation 1 (yeast homolog)-like
M62424	coagulation factor II (thrombin) receptor
M37435	colony stimulating factor 1 (macrophage)
M11220	colony stimulating factor 2 (granulocyte-macrophage)
M92934	connective tissue growth factor
X56692	C-reactive protein, pentraxin-related
D84657	cryptochrome 1 (photolyase-like)
L13278	crystallin, zeta (quinone reductase)
L12579	cut ( <i>Drosophila</i> )-like 1 (CCAAT displacement protein)
U66838	cyclin A1
AF091433	cyclin E2
D88435	cyclin G associated kinase
U47413	cyclin G1

Genbank Accession Number	Name
U47414	cyclin G2
U11791	cyclin H
AF045161	cyclin T1
M68520	cyclin-dependent kinase 2
L25676	cyclin-dependent kinase 9 (CDC2-related kinase)
U17075	cyclin-dependent kinase inhibitor 2B (p15, inhibits CDK4)
L25876	cyclin-dependent kinase inhibitor 3 (CDK2-associated dual specificity phosphatase)
M28668	cystic fibrosis transmembrane conductance regulator, ATP-binding cassette (sub-family C, member 7)
Z00036	cytochrome P450, subfamily I (aromatic compound-inducible), polypeptide 2
J02871	cytochrome P450, subfamily IVB, polypeptide 1
M13267	cytosolic superoxide dismutase 1 (SOD1)
U18321	death associated protein 3
AF015956	death-associated protein 6
X76104	death-associated protein kinase 1
M98331	defensin, alpha 6, Paneth cell-specific
Z71389	defensin, beta 2
M74777	dipeptidylpeptidase IV (CD26, adenosine deaminase complexing protein 2)
M60278	diphtheria toxin receptor (heparin-binding epidermal growth factor-like growth factor)
X74764	discoidin domain receptor family, member 2
AF064019	DNA fragmentation factor, 40 kD, beta polypeptide (caspase-activated DNase)
U91985	DNA fragmentation factor, 45 kD, alpha polypeptide
X59764	DNA-(apurinic or apyrimidinic site) lyase; AP endonuclease 1; APEX nuclease (APEN; APE1); REF-1 protein
D28468	DNA-binding protein TAXREB302; albumin D box-binding protein (DBP)
U35835	DNA-dependent protein kinase (DNA-PK) + DNA-PK catalytic subunit (XRCC7)
D49547	DnaJ (Hsp40) homolog, subfamily B, member 1
U28424	DnaJ (Hsp40) homolog, subfamily C, member 3
L11329	dual specificity phosphatase 2
Y08302	dual specificity phosphatase 9
M25269	ELK1, member of ETS oncogene family
J05081	endothelin 3
L06623	endothelin receptor type B
M18391	EphA1
L41939	EphB2
U14187	ephrin-A3
U14188	ephrin-A4
U12535	epidermal growth factor receptor pathway substrate 8
L05779	epoxide hydrolase 2, cytoplasmic
L16464	ets variant gene 3
M31899	excision repair cross-complementing rodent repair

Genbank Accession Number	Name
	deficiency, complementation group 3 (xeroderma pigmentosum group B complementing)
L04791	excision repair cross-complementing rodent repair deficiency, complementation group 6
X60188	extracellular signal-regulated kinase 1 (ERK1); insulin-stimulated MAP2 kinase; MAP kinase 1 (MAPK 1); p44-MAPK; microtubule-associated protein-2 kinase
X86779	FAST kinase
X52192	feline sarcoma (Snyder-Theilen) viral (v-fes)/Fujinami avian sarcoma (PRCII) viral (v-fps) oncogene homolog
M64082	flavin containing monooxygenase 1
M76673	formyl peptide receptor-like 2
X16706	FOS-like antigen 2
X16707	FOS-like antigen-1
M19922	fructose-1,6-bisphosphatase 1
X15376	gamma-aminobutyric acid (GABA) A receptor, gamma 2
L34357	GATA-binding protein 4
AF067855	geminin
M95809	general transcription factor IIH, polypeptide 1 (62kD subunit)
M64752	glutamate receptor, ionotropic, AMPA 1
D28538	glutamate receptor, metabotropic 5
Y00433	glutathione peroxidase 1
X53463	glutathione peroxidase-gastrointestinal (GSHPX-GI); glutathione peroxidase-related protein 2 (GPRP)
X15722	glutathione reductase
X08020	glutathione S-transferase M4
L33801	glycogen synthase kinase 3 beta
V00518	glycoprotein hormones, alpha polypeptide
X53799	GRO2 oncogene
AF078077	growth arrest and DNA-damage-inducible, beta
AF078078	growth arrest and DNA-damage-inducible, gamma
L29511	growth factor receptor-bound protein 2
U10550	GTP-binding protein overexpressed in skeletal muscle
L22075	guanine nucleotide binding protein (G protein), alpha 13
M14631	guanine nucleotide binding protein (G protein), alpha stimulating activity polypeptide 1
AF017656	guanine nucleotide binding protein (G protein), beta 5
M36430	guanine nucleotide binding protein (G protein), beta polypeptide 1
M36429	guanine nucleotide binding protein (G protein), beta polypeptide 2
X66533	guanylate cyclase 1, soluble, beta 3
X54079	heat shock 27kD protein 1
M11717	heat shock 70kD protein 1A
Y00371	heat shock 70kD protein 8
M64673	heat shock transcription factor 1
X06985	heme oxygenase (decycling) 1
D21243	heme oxygenase (decycling) 2

Genbank Accession Number	Name
M60718	hepatocyte growth factor (hepapoietin A; scatter factor)
X76930	hepatocyte nuclear factor 4, alpha
D16431	hepatoma-derived growth factor (high-mobility group protein 1-like)
D14012	HGF activator
M75952	homeo box 11 (T-cell lymphoma 3-associated breakpoint)
U03056	hyaluronoglucosaminidase 1
AF071596	immediate early response 3
L14754	immunoglobulin mu binding protein 2
M13981	inhibin, alpha
J03634	inhibin, beta A (activin A, activin AB alpha polypeptide)
M97796	inhibitor of DNA binding 2, dominant negative helix-loop-helix protein
X69111	inhibitor of DNA binding 3, dominant negative helix-loop-helix protein
AF044195	inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase complex-associated protein
M10051	insulin receptor
J05046	insulin receptor-related receptor
M27544	insulin-like growth factor 1 (somatomedin C)
X04434	insulin-like growth factor 1 receptor
M29645	insulin-like growth factor 2 (somatomedin A)
M31145	insulin-like growth factor binding protein 1
M31159	insulin-like growth factor binding protein 3
M14648	integrin, alpha V (vitronectin receptor, alpha polypeptide, antigen CD51)
J02703	integrin, beta 3 (platelet glycoprotein IIIa, antigen CD61)
J05633	integrin, beta 5
M73780	integrin, beta 8
U40282	integrin-linked kinase
X14454	interferon regulatory factor 1
M28622	interferon, beta 1, fibroblast
J00209	interferon-alpha2 precursor (IFN-alpha; IFNA); leukocyte interferon-alphaA (LEIF A); roferon + IFN-alpha10 precursor; LEIF C; IFN-alpha-6L
K02770	interleukin 1, beta
M57627	interleukin 10
U03187	interleukin 12 receptor, beta 1
M65291	interleukin 12A (natural killer cell stimulatory factor 1, cytotoxic lymphocyte maturation factor 1, p35)
M74782	interleukin 3 receptor, alpha (low affinity)
X04688	interleukin 5 (colony-stimulating factor, eosinophil)
M75914	interleukin 5 receptor, alpha
M57230	interleukin 6 signal transducer (gp130, oncostatin M receptor)
Y00787	interleukin 8
M68932	interleukin 8 receptor, alpha

Genbank Accession Number	Name
U58198	interleukin enhancer binding factor 1
U10324	interleukin enhancer binding factor 3, 90kD
AF005216	Janus kinase 2 (a protein tyrosine kinase)
U09607	Janus kinase 3 (a protein tyrosine kinase, leukocyte)
AF052432	katanin p80 (WD40-containing) subunit B 1
M74387	L1 cell adhesion molecule (hydrocephalus, stenosis of aqueduct of Sylvius 1, MASA (mental retardation, aphasia, shuffling gait and adducted thumbs) syndrome, spastic paraplegia 1)
X53961	lactotransferrin
X61615	leukemia inhibitory factor receptor
X84740	ligase III, DNA, ATP-dependent
D26309	LIM domain kinase 1
AF036905	linker for activation of T cells
AF055581	lymphocyte adaptor protein
U07236	lymphocyte-specific protein tyrosine kinase
L11015	lymphotoxin beta (TNF superfamily, member 3)
S75313	Machado-Joseph disease (spinocerebellar ataxia 3, olivopontocerebellar ataxia 3, autosomal dominant, ataxin 3)
X70040	macrophage stimulating 1 receptor (c-met-related tyrosine kinase)
U57456	MAD (mothers against decapentaplegic, <i>Drosophila</i> ) homolog 1
U44378	MAD (mothers against decapentaplegic, <i>Drosophila</i> ) homolog 4
M11886	major histocompatibility complex, class I, C
X57766	matrix metalloproteinase 11 (stromelysin 3)
X89576	matrix metalloproteinase 17 (membrane-inserted)
J03210	matrix metalloproteinase 2 (gelatinase A, 72kD gelatinase, 72kD type IV collagenase)
D50477	matrix metalloproteinase-16 precursor (MMP-16); membrane-type matrix metalloproteinase 3 (MT-MMP 3); MMP-X2
L06895	MAX dimerization protein
D21063	MCM2 DNA replication licensing factor (nuclear protein BM28) (KIAA0030).
X82895	membrane protein, palmitoylated 2 (MAGUK p55 subfamily member 2)
J02958	met proto-oncogene (hepatocyte growth factor receptor)
D84557	minichromosome maintenance deficient (mis5, <i>S. pombe</i> ) 6
D38073	minichromosome maintenance deficient ( <i>S. cerevisiae</i> ) 3
X74794	minichromosome maintenance deficient ( <i>S. cerevisiae</i> ) 4
X74795	minichromosome maintenance deficient ( <i>S. cerevisiae</i> ) 5 (cell division cycle 46)
D55716	minichromosome maintenance deficient ( <i>S. cerevisiae</i> ) 7
L26318	mitogen-activated protein kinase 8
U25265	mitogen-activated protein kinase kinase 5
U39657	mitogen-activated protein kinase kinase 6
D14497	mitogen-activated protein kinase kinase kinase 8
U09578	mitogen-activated protein kinase-activated protein kinase

Genbank Accession Number	Name
	3
X72755	monokine induced by gamma interferon
Z12020	mouse double minute 2, human homolog of; p53-binding protein
X76538	MpV17 transgene, murine homolog, glomerulosclerosis
M62397	mutated in colorectal cancers
U07418	mutL (E. coli) homolog 1 (colon cancer, nonpolyposis type 2)
U18840	myelin oligodendrocyte glycoprotein
L08246	myeloid cell leukemia sequence 1 (BCL2-related)
M81750	myeloid cell nuclear differentiation antigen
M33374	NADH-ubiquinone oxidoreductase B18 subunit; complex I-B18 (CI-B18); cell adhesion protein SQM1
M81840	neural retina leucine zipper
L12261	neuregulin 1
X02751	neuroblastoma RAS viral (v-ras) oncogene homolog
U02081	neuroepithelial cell transforming gene 1
M60915	neurofibromin 1 (neurofibromatosis, von Recklinghausen disease, Watson disease)
L11353	neurofibromin 2 (bilateral acoustic neuroma)
U05012	neurotrophic tyrosine kinase, receptor, type 3
M86528	neurotrophin 5 (neurotrophin 4/5)
X96586	neutral sphingomyelinase (N-SMase) activation associated factor
L09210	nitric oxide synthase 2A (inducible, hepatocytes)
L16785	non-metastatic cells 2, protein (NM23B) expressed in
Z11583	nuclear mitotic apparatus protein 1
M24898	nuclear receptor subfamily 1, group D, member 1
X12795	nuclear receptor subfamily 2, group F, member 1
M29971	O-6-methylguanine-DNA methyltransferase
M27288	oncostatin M
U63717	osteoclast stimulating factor 1
U24152	p21/Cdc42/Racl-activated kinase 1 (yeast Ste20-related)
M96944	paired box gene 5 (B-cell lineage specific activator protein)
L19606	paired box gene 8
D13510	pancreatitis-associated protein
M31213	papillary thyroid carcinoma-encoded protein + ret proto-oncogene
M63012	paraoxonase 1
M24398	parathymosin
L19185	peroxiredoxin 2
U92436	phosphatase and tensin homolog (mutated in multiple advanced cancers 1)
U85245	phosphatidylinositol-4-phosphate 5-kinase, type II, beta
U40370	phosphodiesterase 1A, calmodulin-dependent
U56976	phosphodiesterase 1B, calmodulin-dependent
U02882	phosphodiesterase 4D, cAMP-specific (dunce (Drosophila)-

Genbank Accession Number	Name
	homolog phosphodiesterase E3)
Z29090	phosphoinositide-3-kinase, catalytic, alpha polypeptide
U86453	phosphoinositide-3-kinase, catalytic, delta polypeptide
M61906	phosphoinositide-3-kinase, regulatory subunit, polypeptide 1 (p85 alpha)
X80907	phosphoinositide-3-kinase, regulatory subunit, polypeptide 2 (p85 beta)
M34667	phospholipase C, gamma 1 (formerly subtype 148)
X14034	phospholipase C, gamma 2 (phosphatidylinositol-specific)
M54915	pim-1 oncogene
X54936	placental growth factor, vascular endothelial growth factor-related protein
X05199	plasminogen
U08839	plasminogen activator, urokinase receptor
D10202	platelet-activating factor receptor
X02811	platelet-derived growth factor beta polypeptide (simian sarcoma viral (v-sis) oncogene homolog)
U01038	polo (Drosophila)-like kinase
L09561	polymerase (DNA directed), epsilon
U24660	potassium inwardly-rectifying channel, subfamily J, member 6
Y15065	potassium voltage-gated channel, KQT-like subfamily, member 2
AF033347	potassium voltage-gated channel, KQT-like subfamily, member 3
X13403	POU domain, class 2, transcription factor 1
M36542	POU domain, class 2, transcription factor 2
X67055	pre-alpha (globulin) inhibitor, H3 polypeptide
U41816	prefoldin 4
K02268	prodynorphin
S85655	prohibitin
D45027	protease inhibitor 15
D45248	proteasome (prosome, macropain) activator subunit 2 (PA28 beta)
D88378	proteasome (prosome, macropain) inhibitor subunit 1 (PI31)
D00759	proteasome (prosome, macropain) subunit, alpha type, 1
D00762	proteasome (prosome, macropain) subunit, alpha type, 3
D00763	proteasome (prosome, macropain) subunit, alpha type, 4
S76965	protein kinase (cAMP-dependent, catalytic) inhibitor alpha
J03075	protein kinase C substrate 80K-H
X65293	protein kinase C, epsilon
M55284	protein kinase C, eta
U33053	protein kinase C-like 1
M34182	protein kinase, cAMP-dependent, catalytic, gamma
M33336	protein kinase, cAMP-dependent, regulatory, type I, alpha (tissue specific extinguisher 1)
X14968	protein kinase, cAMP-dependent, regulatory, type II, alpha

Genbank Accession Number	Name
M31158	protein kinase, cAMP-dependent, regulatory, type II, beta
M35663	protein kinase, interferon-inducible double stranded RNA dependent
M63960	protein phosphatase 1, catalytic subunit, alpha isoform
S87759	protein phosphatase 1A (formerly 2C), magnesium-dependent, alpha isoform
X12646	protein phosphatase 2 (formerly 2A), catalytic subunit, alpha isoform
M64929	protein phosphatase 2 (formerly 2A), regulatory subunit B (PR 52), alpha isoform
M64930	protein phosphatase 2 (formerly 2A), regulatory subunit B (PR 52), beta isoform
L14778	protein phosphatase 3 (formerly 2B), catalytic subunit, alpha isoform (calcineurin A alpha)
M30773	protein phosphatase 3 (formerly 2B), regulatory subunit B (19kD), alpha isoform (calcineurin B, type I)
U48296	protein tyrosine phosphatase type IVA, member 1
L08807	protein tyrosine phosphatase, non-receptor type 11
L09247	protein tyrosine phosphatase, receptor type, G
M93426	protein tyrosine phosphatase, receptor-type, Z polypeptide 1
M35203	protein-tyrosine kinase (JAK1)
L34583	protein-tyrosine phosphatase 1E
M26708	prothymosin, alpha (gene sequence 28)
L13616	PTK2 protein tyrosine kinase 2
U33635	PTK7 protein tyrosine kinase 7
Y07701	PUROMYCIN-SENSITIVE AMINOPEPTIDASE (EC 3.4.11.-) (PSA)
D10923	putative chemokine receptor; GTP-binding protein
L42450	pyruvate dehydrogenase kinase, isoenzyme 1
L42379	quiescin Q6
M28210	RAB3A, member RAS oncogene family
M28211	RAB4, member RAS oncogene family
M28215	RAB5A, member RAS oncogene family
M28212	RAB6A, member RAS oncogene family
U63139	RAD50 (S. cerevisiae) homolog
X82260	Ran GTPase activating protein 1
M64788	RAP1, GTPase activating protein 1
X63465	RAP1, GTP-GDP dissociation stimulator 1
M22995	RAP1A, member of RAS oncogene family
X08004	RAP1B, member of RAS oncogene family
X06820	ras homolog gene family, member B
M23379	RAS p21 protein activator (GTPase activating protein) 1
L26584	Ras protein-specific guanine nucleotide-releasing factor 1
L24564	Ras-related associated with diabetes
M29870	ras-related C3 botulinum toxin substrate 1 (rho family, small GTP binding protein Rac1)
X93499	ras-related protein RAB-7

Genbank Accession Number	Name
M97675	receptor tyrosine kinase-like orphan receptor 1
D10232	renin-binding protein
L07541	replication factor C (activator 1) 3 (38kD)
M87339	replication factor C (activator 1) 4 (37kD)
L07493	replication protein A3 (14kD)
M15400	retinoblastoma 1 (including osteosarcoma)
S66431	retinoblastoma-binding protein 2
X74594	retinoblastoma-like 2 (p130)
X07282	retinoic acid receptor, beta
M84820	retinoid X receptor, beta
U17032	Rho GTPase activating protein 5
U02082	Rho guanine nucleotide exchange factor (GEF) 5
X56932	ribosomal protein L13a
X69391	ribosomal protein L6
L07597	ribosomal protein S6 kinase, 90kD, polypeptide 1
U23946	RNA binding motif protein 5
X58079	S100 calcium-binding protein A1
M86757	S100 calcium-binding protein A7 (psoriasin 1)
X06234	S100 calcium-binding protein A8 (calgranulin A)
U01160	sarcoma amplified sequence
Y00757	secretory granule, neuroendocrine protein 1 (7B2 protein)
U60800	sema domain, immunoglobulin domain (Ig), transmembrane domain (TM) and short cytoplasmic domain, (semaphorin) 4D
M14091	serine (or cysteine) proteinase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 7
U04313	serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 5
L40377	serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 8
U71364	serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 9
X04429	serine (or cysteine) proteinase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 1
Z81326	serine (or cysteine) proteinase inhibitor, clade I (neuroserpin), member 1
U78095	serine protease inhibitor, Kunitz type, 2
AF008552	serine/threonine kinase 12
L20321	serine/threonine kinase 2
D84212	serine/threonine kinase 6
M97935	signal transducer and activator of transcription 1, 91kD
M97934	signal transducer and activator of transcription 2 (STAT2); p113
L29277	signal transducer and activator of transcription 3 (acute-phase response factor)
M57502	small inducible cytokine A1 (I-309, homologous to mouse Tca-3)
J04130	small inducible cytokine A4 (homologous to mouse Mip-1b)
M21121	small inducible cytokine A5 (RANTES)
AJ002211	small inducible cytokine B subfamily (Cys-X-Cys motif), member 13 (B-cell chemoattractant)

Genbank Accession Number	Name
U46767	small inducible cytokine subfamily A (Cys-Cys), member 13
X02530	small inducible cytokine subfamily B (Cys-X-Cys), member 10
X78686	small inducible cytokine subfamily B (Cys-X-Cys), member 5 (epithelial-derived neutrophil-activating peptide 78)
U10117	small inducible cytokine subfamily E, member 1 (endothelial monocyte-activating)
M21940	S-mephenytoin 4 hydroxylase; cytochrome P450 IIC9 (CYP2C9) + CYP2C10 + CYP2C17 + CYP2C18 + CYP2C19
AF068920	soc-2 (suppressor of clear, <i>C.elegans</i> ) homolog
M77235	sodium channel, voltage-gated, type V, alpha polypeptide (long (electrocardiographic) QT syndrome 3)
D00099	SODIUM/POTASSIUM-TRANSPORTING ATPASE ALPHA-1 CHAIN (EC 3.6.1.37) (SODIUM PUMP) (NA+/K+ ATPASE).
L14595	solute carrier family 1 (glutamate/neutral amino acid transporter), member 4
U03506	solute carrier family 1 (neuronal/epithelial high affinity glutamate transporter, system Xag), member 1
U13173	solute carrier family 15 (oligopeptide transporter), member 1
L31801	solute carrier family 16 (monocarboxylic acid transporters), member 1
U10554	solute carrier family 18 (vesicular acetylcholine), member 3
L09118	solute carrier family 18 (vesicular monoamine), member 2
U14528	solute carrier family 26 (sulfate transporter), member 2
AF025409	solute carrier family 30 (zinc transporter), member 4
M95549	solute carrier family 5 (sodium/glucose cotransporter), member 2
M95167	solute carrier family 6 (neurotransmitter transporter, dopamine), member 3
S75989	solute carrier family 6 (neurotransmitter transporter, GABA), member 11
S70609	solute carrier family 6 (neurotransmitter transporter, glycine), member 9
S80071	solute carrier family 6 (neurotransmitter transporter, L-proline), member 7
AJ000730	solute carrier family 7 (cationic amino acid transporter, y+ system), member 4
AF077866	solute carrier family 7 (cationic amino acid transporter, y+ system), member 5
M81768	solute carrier family 9 (sodium/hydrogen exchanger), isoform 1 (antiporter, Na+/H+, amiloride sensitive)
L13857	son of sevenless ( <i>Drosophila</i> ) homolog 1
M97190	Sp2 transcription factor
AF039843	sprouty ( <i>Drosophila</i> ) homolog 2
U08098	sulfotransferase, estrogen-preferring
AF069734	suppressor of Ty ( <i>S.cerevisiae</i> ) 3 homolog
AF046873	synapsin III
X07024	TATA box binding protein (TBP)-associated factor, RNA

Genbank Accession Number	Name
	polymerase II, A, 250kD
D29767	tec protein tyrosine kinase
M16552	thrombomodulin
M92381	thymosin, beta 10
M17733	thymosin, beta 4, X chromosome
U76456	tissue inhibitor of metalloproteinase 4
X69490	titin
J03250	topoisomerase (DNA) I
U59863	TRAF family member-associated NFkB activator
M80627	transcription factor 12 (HTF4, helix-loop-helix transcription factors 4)
M36711	transcription factor AP-2 alpha (activating enhancer-binding protein 2 alpha)
L23959	transcription factor Dp-1
U18422	transcription factor Dp-2 (E2F dimerization partner 2)
AF009353	transcriptional intermediary factor 1
J03241	transforming growth factor, beta 3
L07594	transforming growth factor, beta receptor III (betaglycan, 300kD)
X95384	translational inhibitor protein p14.5
U78773	tripartite motif-containing 28
U04811	trophinin
X52836	tryptophan hydroxylase (tryptophan 5-monooxygenase)
X75621	tuberous sclerosis 2
U57059	tumor necrosis factor (ligand) superfamily, member 10
D38122	tumor necrosis factor (ligand) superfamily, member 6
X01394	tumor necrosis factor (TNF superfamily, member 2)
M32315	tumor necrosis factor receptor superfamily, member 1B
M14694	tumor protein p53 (Li-Fraumeni syndrome)
U82130	tumor susceptibility gene 101
D17517	TYRO3 protein tyrosine kinase
X57346	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, beta polypeptide
L20422	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, eta polypeptide
M26880	ubiquitin C
M32977	vascular endothelial growth factor
U43142	vascular endothelial growth factor C
V00574	v-Ha-ras Harvey rat sarcoma viral oncogene homolog
AF055377	v-maf musculoaponeurotic fibrosarcoma (avian) oncogene homolog
J00119	v-mos Moloney murine sarcoma viral oncogene homolog
M15024	v-myb avian myeloblastosis viral oncogene homolog
X66087	v-myb avian myeloblastosis viral oncogene homolog-like 1
X13293	v-myb avian myeloblastosis viral oncogene homolog-like 2
V00568	v-myc avian myelocytomatosis viral oncogene homolog
L15409	von Hippel-Lindau syndrome
X03484	v-raf-1 murine leukemia viral oncogene homolog 1
X15014	v-ras simian leukemia viral oncogene homolog A (ras)

Genbank Accession Number	Name
	related)
M35416	v-ras simian leukemia viral oncogene homolog B (ras related; GTP binding protein)
X75042	v-rel avian reticuloendotheliosis viral oncogene homolog
L19067	v-rel avian reticuloendotheliosis viral oncogene homolog A (nuclear factor of kappa light polypeptide gene enhancer in B-cells 3 (p65))
M34353	v-ros avian UR2 sarcoma virus oncogene homolog 1
M16038	v-yes-1 Yamaguchi sarcoma viral related oncogene homolog
U10564	wee1+ (S. pombe) homolog
X51630	Wilms tumor 1
Z71621	wingless-type MMTV integration site family, member 2B
D21089	xeroderma pigmentosum, complementation group C
M36089	X-ray repair complementing defective repair in Chinese hamster cells 1
M30938	X-ray repair complementing defective repair in Chinese hamster cells 5 (double-strand-break rejoining; Ku autoantigen, 80kD)
M76541	YY1 transcription factor
D26121	ZFMI protein alternatively spliced product
M28372	zinc finger protein 9 (a cellular retroviral nucleic acid binding protein)
X59738	zinc finger X-chromosomal protein (ZFX)
X94991	zyxin

Throughout this application various patent and non-patent publications have been referenced. The disclosures of these publications in their entireties are 5 hereby incorporated by reference in this application in order to more fully describe the state of the art to which this invention pertains.

The term "comprising" is intended herein to be 10 open-ended, including not only the recited elements, but further encompassing any additional elements.

Although the invention has been described with reference to the examples provided above, it should be 15 understood that various modifications can be made without

departing from the spirit of the invention. Accordingly, the invention is limited only by the claims.